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REVIEW ARTICLE

# Current Status of Noninvasive Prenatal Testing and Counselling Considerations: An Indian Perspective

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**Abstract** Uptake of noninvasive prenatal testing (NIPT) is rapidly expanding around the world. Here, we provide an overview of the current global state of NIPT, describe the expansion of the test menu, highlight alternative prenatal test service delivery, and discuss NIPT counseling considerations. We also provide a perspective on utilisation of NIPT in India, which has unique challenges for implementing NIPT given its large population, vast territory, and diverse ethnic groups. The barriers to implementation of NIPT in India are also discussed. Current recommendations regarding use of NIPT made by professional societies vary in different regions and such recommendations for NIPT in India will be helpful to provide general guidance to the health care providers, but will likely require modifications for implementation in India.

**Keywords** Noninvasive prenatal testing (NIPT) · Noninvasive prenatal screening (NIPS) · Cell-free DNA (cfDNA) · India · Counselling · Prenatal Diagnosis Techniques Act · Limitations · Guidelines

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## Introduction

While the discovery of fetal cell-free DNA (cfDNA) in maternal plasma occurred in 1997 [1], noninvasive prenatal testing (NIPT) for fetal aneuploidy based on analysing circulating cfDNA in maternal plasma was not commercially available until 2011 [2, 3]. Currently, NIPT is available in over 60 countries [4], including India. There are 26 million births per year in India with an incidence of trisomy 21 being 1/1150 in liveborn at birth [5]. Introduction of NIPT into the prenatal screening paradigm occurred rapidly in many countries and was not surprising given its lower false-positive rate (FPR) and higher positive predictive value across all risk profiles, when compared to standard aneuploidy screening [6]. NIPT is one of the few tests that not only has shown rapid adoption in clinical practice, but also an expansion in testing menu from screening for common chromosomal anomalies such as trisomy 21,18,13 to screening for a wide variety of genome-wide chromosomal anomalies [7, 8].

There are currently several prenatal testing options available for patients that would like further information about their pregnancy with regards to presence of fetal aneuploidy. These include invasive diagnostic tests, such as chorionic villus sampling (CVS) and amniocentesis, which carry a risk of miscarriage [9]. There are also noninvasive screening tests such as combined first trimester screening or other maternal serum screens such as triple marker or quad marker serum testing that screen for Down syndrome and other common aneuploidies. However, these noninvasive screening tests have false-positive rates of around 5% [6, 10]. In contrast, NIPT has demonstrated a FPR of 0.04% for trisomy 21 [11]. Given this lower FPR and the higher positive predictive value, NIPT is offered in many countries, either as a private pay or is integrated into

national healthcare systems. As NIPT is incorporated into the prenatal screening paradigm, and the test menus expand, there will be a need for appropriate counseling information.

## Global NIPT Implementation

After NIPT became commercially available in 2011, implementation around the globe varied. In the United States, adoption of NIPT for the common trisomies in high-risk populations occurred rapidly and was supported by professional societies [12]. With support from professional societies, private insurers as well as state funded insurers began to reimburse the cost of NIPT, and this set the stage for further adoption.

Concurrently, NIPT expanded as a cash test world-wide, especially in Europe and even in some Asian countries. However, the cash pay model leads to disparities in patient access. For instance, the nationalized healthcare system in Australia does not currently cover reimbursement for NIPT, despite supportive professional guidelines for use of NIPT in screening. As expected, this has led to significant disparities in access among different socioeconomic groups [13, 14].

In addition, several countries with large public health systems conducted large scale studies, as required, to show the clinical value of NIPT and how it could be implemented on a population-wide scale. The studies conducted varied by country given each country had to address their unique questions and challenges that would be faced by patients and providers as well as the overall impact on the health care system.

One such study in the Netherlands, the TRIDENT program for offering NIPT, was initiated in 2014 in response to the growing demand from patients and healthcare providers. The goals of the program were to responsibly implement NIPT into the healthcare pathway to ensure the quality of testing, equal accessibility to patients, as well as access to appropriate counselling support. Initially, NIPT was offered on a contingent basis to high-risk groups using a 1/200 first-trimester combined test cut-off or a medical history suggestive of increased risk [15]. Given the success of the initial program, TRIDENT 2 was initiated in April 2017, and NIPT is now offered to all pregnant women.

Another European study is known as the RAPID study, which was performed in UK in 2013, focused entirely on the health economic aspect of NIPT implementation. This study revealed that offering NIPT contingently using a trisomy 21 cut-off of 1 in 150 on first trimester combined screening (FCT) would result in overall cost reduction compared to the current screening program [16]. The UK National Screening Committee (NSC) now recommends nationwide implementation of a contingent NIPT model based upon this risk threshold [17].

First trimester combined screening is widely used for prenatal screening for trisomies 21, 18, and 13, and many countries, especially those in Europe, have adopted a contingent screening model to identify high risk pregnant patients who should be offered NIPT. France, Belgium, Czech Republic, Denmark, Switzerland and now have national funding. In Canada, Italy, Spain, and China funding is only available in certain regions. In addition, there are different FCT cut-offs that are used to determine eligibility. The cut-offs vary from 1/150 in UK to 1/1000 in France. These differences are most likely based on the -health economic model of the nationalized health systems.

Meanwhile in many developing nations, adoption of NIPT has remained low. There are several possible reasons for this including a current state of prenatal screening and testing, lack of professional society guidelines, socio-economic factors, cost of the test compared to the gross domestic product, and lack of physician knowledge. Although India has no nationalized or regional health care systems, the challenges faced in introducing NIPT will be somewhat similar to those encountered in other nations.

## Expansion of the NIPT Menu

Since its global introduction, there has been a progressive expansion of the NIPT menu. The initial offering of NIPT included screening for common autosomal trisomies in singleton pregnancies, namely trisomy 21, 18, or 13, with superior test performance observed compared to other prenatal screening tests [18]. NIPT testing was further expanded to sex chromosome aneuploidies such as Turner syndrome (XO), XXX, XXY and XYY [19, 20]. Still, limiting the menu to the common trisomies and sex chromosome aneuploidies will miss an additional 16% of chromosomal anomalies [21]. Over the past few years, given the genome-wide analysis capability of some of the NIPT offerings, the screened conditions were expanded to include other chromosomal anomalies such as rare autosomal aneuploidies (RAAs) and partial deletions and duplications $\geq$ 7 Mb across the genome [22–26], as well as select microdeletion panels. This resolution is roughly equivalent to resolution on conventional prenatal karyotype. Although these conditions are not as prevalent as the common aneuploidies, presently these conditions cannot be screened for using the current screening modalities. In addition, NIPT has also been expanded to include screening for common aneuploidies in twin pregnancies [27, 28] with superior performance compared to other prenatal screening tests [29].

Rare autosomal aneuploidies in NIPT occur at a rate of around 0.34% [8]. The screen-positive rate of partial

deletions and duplications has been shown to be around 0.1% [25, 30, 31]. While the utility of screening for genome-wide imbalances outside of the common trisomies and sex chromosome aneuploidies is still being established, there is growing evidence to support clinical utility. Patients that receive an NIPT result positive for these rare chromosomal anomalies may have an up to a 75% increased risk for miscarriage, intrauterine growth restriction (IUGR), intrauterine fetal demise, or fetal anomalies due to true fetal mosaicism, and uniparental disomy (UPD) [8, 22, 23, 26, 30]. Although some of the rare autosomal aneuploidies identified on NIPT may be confined to the placenta, identifying these cases is still of clinical relevance as they can lead to pregnancy complications such as IUGR.

Some NIPT offerings also include select microdeletion screening panels [32–34]. The conditions included in the microdeletion panel were based on clinical severity of the phenotype, the incidence of the condition, and technical feasibility of screening for these conditions. These include conditions such as 22q11 deletion syndrome (DiGeorge or Velocardiofacial syndrome), 1p36 deletion syndrome, 4p deletion syndrome (Wolf-Hirschhorn syndrome), 5p deletion syndrome (Cri-du-chat syndrome), and 15q11.2 deletion syndrome (Angelman and Prader-Willi syndromes). There are certain NIPT tests that can screen for other microdeletions and microduplications.

Detection of single-gene conditions is also possible with cell-free DNA (cfDNA) approaches. Although not widely used, the UK has specifically utilized cfDNA testing for diagnosis of autosomal recessive disorders where the parents have known mutations and autosomal dominant de novo disorders.

## The Indian Perspective

Noninvasive prenatal testing was introduced in India in December 2012 by three providers. Blood samples were collected in Streck tubes and shipped to the USA or China. By June 2014, at Sir Ganga Ram Hospital, we had carried out NIPT in 500 women who mostly had positive contingent screening tests or had soft markers detected on ultrasound studies [35]. Pre- and post-test counselling was offered to all women, explaining that NIPT is a high efficiency screening test, but not a diagnostic test. In 2015 a 10-center study using SNPs was initiated on a research basis, and the samples were processed and analysed in India [36]. This study provided valuable experience to Indian obstetricians and fetal medicine specialists. Of the 511 samples analysed, results were obtained in 499 (97.7%). 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 the abnormalities tested. Overall the results were similar to those observed in previous studies carried out in other countries [18, 37, 38]. The average fetal fraction was 8.2%, which was higher than the average observed in the West, mainly due to a lower BMI of Indian women. Verma et al. examined the adaptations that may be required to the ACMG guidelines on NIPT for use in India [39, 40].

### The Prenatal Diagnostic Techniques (Regulation and Prevention of Misuse) Act, 1994

The Prenatal Diagnostic Techniques Act was introduced on 1st January 1996 to control for female feticide [41, 42]. It prohibits determination and disclosure of the sex of the fetus, and also prohibits any advertisements relating to prenatal determination of sex. An amendment in January 2003 banned the use of sex-selection techniques before and after conception and included IVF and other assisted reproduction technologies [43]. It outlines how prenatal diagnostic techniques should not be conducted except for the purposes of detection of one of the following abnormalities, namely: (i) chromosomal abnormalities; (ii) genetic metabolic diseases; (iii) haemoglobinopathies; (iv) sex-linked genetic diseases; (v) congenital anomalies; (vi) any other abnormalities or diseases as may be specified by the Central Supervisory Board; the Act allows prenatal diagnosis when there is an X-linked disease, but the law does not clarify whether the sex of the fetus can be disclosed in this instance. It further states that no prenatal diagnostic techniques shall be used or conducted unless the person qualified to do so is satisfied for reasons to be recorded in writing that any of the following conditions are fulfilled: (i) age of the pregnant woman is above 35 years, (ii) she has undergone two or more spontaneous abortions or fetal losses, (iii) she has been exposed to potentially teratogenic agents, such as drugs, radiation, infections or chemicals, (iv) the pregnant woman or her spouse has a family history of mental retardation or physical deformities such as spasticity, or any other genetic disease. Subsequent amendments made essential the registration of every ultrasound machine, to the extent that the use of ultrasound for the management of pregnancy was hindered. The ultrasonologists made a representation to the Government for removing these amendments to facilitate its legitimate use [44–46].

This Act has hindered the expansion of NIPT testing options in India. In countries where fetal sexing is permitted, diagnosis of sex on cell-free DNA is advantageous for families with an X-linked disease as it allows invasive testing to be avoided in cases where the fetus is female. This simplifies prenatal diagnosis in common X-linked disorders such as Duchenne muscular dystrophy and

haemophilia A and B. Unfortunately, in India determination of fetal sex is forbidden by law so one cannot use this technology for this purpose. Verma [39] calculated that if fetal sexing was permitted in India and carried out using cfDNA in X-linked diseases, there would be a saving of Rs. 5,000,000 per 1000 at risk pregnant women tested.

As far as the Act is concerned it permits the use of NIPT for diagnosis of aneuploidies and sex-linked disorders, with non-disclosure of sex. However, it has forbidden direct marketing to the patient, and allows its use by only those obstetricians and fetal medicine experts that are registered with the relevant authority for carrying out prenatal diagnosis.

### Need for Better Awareness and Knowledge

It is important that both patients and healthcare professionals in India are educated regarding the use of NIPT. The majority of patients in India are ill-informed about NIPT, and the level of knowledge varies amongst obstetricians around the country. Obstetricians in the cities are typically well acquainted with NIPT, although they are more familiar with invasive techniques such as amniocentesis as these have been in use for a long time. However, most of the obstetricians in the peripheral areas have little knowledge about NIPT and there is a need for the providers of this technology to help expand educational efforts in various parts of the country.

### Prohibitive Cost

The majority of women in India pay for NIPT and invasive tests from out-of-pocket expenses, as there is no public funding or insurance coverage for these tests. Most vendors in India offer the NIPT test for aneuploidy of five chromosomes (21, 18. 13, X and Y) for about Rs. 20,000, which is equivalent to US $285, with correspondingly greater charges for expanded testing options such as micro-deletions or other chromosomes. For many patients, this cost is too high and they are unable to pay for it. In addition, insurance companies either do not cover the cost of outpatient investigations during pregnancy or will cover it only if the patient is admitted to the hospital. It is hoped that with improvements in technology and increasing competition the cost will come down allowing increased accessibility to NIPT.

### No Result or Delay in Obtaining Result and Its Consequences

Only a few providers perform the test in India, while others collect the samples and send these to laboratories abroad. This leads to a delay in turn-around times. In addition, the possibility of no result or the need to repeat the test due to low fetal fraction or other causes has to be kept in mind while ordering the test, as it may cause further delay in obtaining results. On the other hand, the number of samples rejected due to a low fetal fraction is less in India as compared to the West, due to a lower BMI of the pregnant women [36]. Collection of a repeat sample is easy if the patient lives in a metropolitan city, but becomes difficult if the patient comes from a peripheral area. For such patients most experts opt for an invasive test instead of NIPT so that patients only have to come in for prenatal testing on one occasion.

### National Program for Aneuploidy Screening and Diagnosis

None of the states in India have a government funded program for aneuploidy detection through biochemical and ultrasound screening, chorionic villus sampling, amniocentesis, or NIPT. At present the states do not consider screening for genetic disorders a priority. The Government has initiated a very ambitious and large-scale program to screen for nutritional deficiencies, disabilities such as deafness and loss of vision, and genetic conditions in children from birth to 18 years. Hopefully, in future there will be a focus on detecting and managing birth defects and genetic disorders of the fetus through screening tests during pregnancy.

### National Consensus for Prenatal Testing

Currently there is no consensus at the national level regarding prenatal testing strategy. Phadke and colleagues suggested that NIPT should be offered to ‘precious pregnancies,’ but there was no clear definition regarding what constitutes a ‘precious pregnancy’ [47]. Phadke et al. further stated that it is difficult to follow a single nationwide protocol due to regional variability in medical services and patient economic status. We disagree with this view and feel that it should be possible to develop a protocol for using NIPT in India taking into consideration the local constraints. In our opinion, the test should not be offered directly to the patients. What is essential is pre- and post-test counselling. The patient must be informed that the basic NIPT test will only return a result for aneuploidies of five chromosomes, and that abnormalities due to the other chromosomes or other causes will not be tested. Some providers do offer extended NIPT screening for other aneuploidies, and this option will require modifications in patient counselling. The final report will only state that the result is low risk or high risk for aneuploidy, and will not state that the chromosomes are ‘normal’. Patients often believe that a “normal” NIPT test result will ensure the

birth of a normal baby. Patients should be informed that NIPT is a screening test only and negative results do not eliminate the possibility of a fetal abnormality. Those patients with positive results should be offered diagnostic testing. The ideal time to offer NIPT is at the first prenatal visit after 10 weeks of gestation, which will maximize the time for patient deliberation regarding management options in the event of positive results. Irreversible medical actions should only be considered once the diagnosis of an abnormality is confirmed via diagnostic testing. Possible false positives due to placental mosaicism or other biologic factors should be kept in mind. In addition, the obstetrician should be clearly informed and familiar with the testing options of the NIPT test available through a particular test provider. For example, NIPT for twins is available through some providers, while not from others. Similarly, only some tests can check for triploidy and vanishing twins. The obstetrician should be also be familiar with the expanded options available through certain providers.

The test should not be used for cases with recurrent miscarriages (unless parental chromosomes are normal), family history of genetic disease or intellectual disability, ultrasound result showing malformations, or increased nuchal translucency or nuchal fold thickness. These latter situations require a full fetal karyotype or preferably a micro-array study.

## Counselling Considerations

When provided with options, patients need help in making decisions. Many professional societies have stressed the importance of shared informed decision-making when providing counselling on aneuploidy screening and testing in pregnancy [12, 48–50]. Ideally, counselling regarding prenatal testing options should be provided at the first prenatal visit, which will maximize the patient's options for testing [51]. Pre-test counselling discussion points should include the patient's specific risk of fetal aneuploidy and testing options available in the patient's local setting [51]. The patient should be provided with accurate and balanced information on the specific conditions being screened [48]. Patients should be aware that NIPT is a screening test; as such, a positive NIPT result needs to be confirmed by prenatal invasive testing prior to making any pregnancy management decisions or by postnatal testing. Specific to NIPT, the patient also should be informed of the potential to detect maternal chromosomal aberrations and other unanticipated maternal findings [49]. The information and counselling provided should be tailored to the patient's level of understanding with consideration regarding what she will choose to do with this information. Ultimately, every patient has the choice of whether or not to proceed with prenatal screening/testing. Lastly, parallel or simultaneous testing with multiple screening methodologies for aneuploidy may lead to conflicting results, will increase unnecessary costs to the patient, and should not be performed [12]. The importance of quality counselling is highlighted by studies showing that patients making an informed choice experience positive psychological effects, including less decisional conflict [52, 53].

There are several points to consider regarding post-test NIPT counselling [12, 48–50]. Firstly, a patient with an NIPT result positive for aneuploidy should be offered additional counselling and an option of diagnostic testing, such as CVS or amniocentesis during pregnancy. This is necessary as NIPT can have false-positive results due to either biological or technical reasons. It is also important to emphasize that no irreversible pregnancy management decisions should be made based on NIPT results alone. In addition, patients should be reminded that NIPT does not have 100% specificity and also screens for limited chromosomal anomalies, and hence a negative screening result does not guarantee a 'normal' outcome. Finally, positive and negative NIPT results should be considered in the context of all available clinical findings. Counselling after a technical test failure or 'no-call' is also important. Failure rates and reasons will depend on the specific NIPT technology used. Failures specifically due to low fetal fraction have been associated with an increased risk for aneuploidies such as trisomy 18 [54]. Those patients who experience test failures due to low fetal fraction should be counselled about their increased risk for aneuploidy; they should also be offered alternative testing including diagnostic testing [55].

Cell-free DNA from maternal plasma is predominantly maternal in origin with an average of 10–20% [56] arising from apoptotic trophoblastic cells. As such, discordance between diagnostic testing and NIPT has been associated with several biological factors, including confined placental mosaicism, true fetal mosaicism, vanishing twin, maternal mosaicism, maternal copy number variation, maternal malignancy, and history of maternal organ transplant, [57]. When discordance between NIPT and diagnostic testing occurs, clinicians should consider following up with the NIPT test provider to discuss the patient history to help determine possible reason for discordance as this information may impact clinical management.

Pre-test and post-test counselling of NIPT should be performed by health care professionals knowledgeable in these areas. Given many obstetricians in India are not familiar with the nuances of NIPT, education on counselling, information-giving, and biologic aspects of NIPT is necessary. This education will require concerted efforts from the Indian OBGYN society as well as test providers.

### Counselling Service Models and Educational Supplements to Consider in India

As mentioned above, the aim of counselling in the context of NIPT is to give the patient the ability to make an informed decision and choice regarding NIPT and all available prenatal testing options. In some parts of the world, counselling is provided on one to one basis by certified genetic counsellors. However, these healthcare professionals are not always easily accessible and available in every country. In these instances, the information may be provided by other knowledgeable healthcare professionals, such as physicians or other healthcare providers.

As genetic testing services expand into India's prenatal setting, there will be a need to develop trained genetic counsellors. Meanwhile, this function can be carried out by obstetricians or allied health professionals with an appropriate education. As one to one counselling may not be always possible given India's large population, alternative forms of providing information and counselling should be considered [58]. The goal of implementing these alternatives would be to provide quality education and counselling, while also expanding patient access and improving service delivery efficiency beyond the capabilities of the traditional in-person approach. With regards to information giving, written materials may be used, or the materials may be provided in other formats such as specific counselling apps and appropriate educational videos (Table 1).

Providing information to patients prior to testing encourages patient participation in the decision-making process and helps to incorporate patient and family values when deciding about test options. This also will help decrease physician time repeating the same information, and the physician can focus on the specific issues at hand [58, 59].

Telegenetic services are becoming more popular and help to provide specialized information to remote areas. Although the technology will need to be available, telegenetic services, including real-time video conferencing, can be an option to assist in providing accessibility to information about NIPT. Studies have consistently shown that patients are satisfied with telegenetic services and found similar satisfaction ratings between telegenetic services and in-person counselling [52, 60]. While not necessarily increasing the number of visits that a given healthcare provider can see in 1 day, telegenetic services provided patients with access to services that otherwise may not be available in their region. In the absence of availability of telegenic equipment, counseling can also be provided via the telephone. An alternative approach in centres with a large volume of referrals is group counselling. It has been shown to decrease patient anxiety, increase perceived control, decrease decisional conflict, and increase knowledge in patients [53, 61]. Also, in a randomized trial comparing these various approaches to prenatal counselling, those who had group counselling demonstrated the greatest increase in knowledge, those who received individual counselling was most satisfied, and those women who used a decision aid had the least decisional conflict. All participants showed a significant increase in knowledge and a decrease in decisional conflict with these various approaches to counselling [62].

## Conclusions

NIPT is a screening test that has seen fast global adoption. However, challenges to access still remain in many parts of the world, including India. While cost remains the major factor, obstetrician and patient education about NIPT is also a barrier. India is a large nation in size, population, uniformity of medical services, and cultural diversity. Given the uniqueness of India, careful consideration of all aspects of NIPT offerings will be necessary. In addition,

**Table 1** Counselling resources

| Resource category | Details | Link |
|---|---|---|
| *Educational supplement* | | |
| Counselling Aid | | https://www.illumina.com/content/dam/illumina-marketing/documents/clinical/rgh/flipbook/counseling-guide-reproductive-genetics-flipbook.pdf |
| UK National Health Service Fetal Anomaly Programme Handbook | | https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/749742/NHS_fetal_anomaly_screening_programme_handbook_FINAL1.2_18.10.18.pdf |
| The Netherlands Brochure on Prenatal Testing Options | | https://www.rivm.nl/sites/default/files/2018-11/101908_009545_GH_Down_EN_TG_NW2.pdf |
| Lettercase.org | Digital and print resources for genetic conditions | https://www.lettercase.org/ |

appropriate guidelines from Indian ObGYN society will help to provide uniform guidance to the Obstetricians in the use of NIPT.

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**Compliance with Ethical Standards**

**Conflict of interest** JM is an employee of Illumina, Inc. VA, ML, SB, RDP and ICV are employees of Institute of Genetics and Genomics, Sir Ganga Ram Hospital, New Delhi.

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