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REVIEW ARTICLE

Noninvasive Prenatal Testing: The Indian Perspective

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Abstract This article summarizes the Professor Kamal Buckshee Oration given by the author at the annual conference of the Society of Fetal Medicine in Kochi, August 2014. The contributions of Professor Buckshee to fetal medicine are briefly described. The article traces the development of fetal medicine in India. It discusses the application of noninvasive prenatal testing (NIPT) in India. It briefly calculates the cost benefits of sexing of the fetus for X-linked disease. It also describes the results of screening 320 pregnant women using NIPT for aneuploidies. The benefits and limitations of NIPT are presented.

Keywords Fetal medicine in India · NIPT · NIPD · X-linked disease · Aneuploidies · Chromosomal disorders

It is an honor and a privilege to be asked to deliver the Professor Kamal Buckshee Oration of the Society of Fetal Medicine. She laid the foundations of fetal medicine in India and pioneered many fetal techniques, when she served as Chairman of the Department of Obstetrics and Gynecology of the All India Institute of Medical Sciences. She was the first Indian obstetrician to carry out the in utero fetal skin biopsy, cordocentesis for fetal diagnosis, and in utero blood transfusion. In the field of gynecology, she evolved the uterine balloon therapy for dysfunctional uterine bleeding. In recognition of her significant contributions she was elected as President of The Federation of Obstetricians and Gynecologists Society of India in 1995, and also received the Lifetime Achievement Award. She has been the recipient of numerous honors, to mention a few—the Dr B.C. Roy Award for being an "Eminent Medical Teacher", Woman of the year 1998 Award, Fellowship of the Royal College of Obstetricians and Gynecologists in the UK, and the National Academy of Medical Sciences in India. She has served as adviser in obstetrics and gynecology to the Indian Council of Medical Research and WHO. She is currently a senior consultant at Fortis La Femme, and Apollo Hospital, New Delhi, and an Emeritus Professor of the National Academy of Medical Sciences.

I was closely associated with her, while she was at the All India Institute of Medical Sciences. We published five papers together—on cordocentestis [1], nomograms for ventricular size [2], and nuchal thickness [3], chorionic villus sampling [4], and prenatal diagnosis of genetic diseases [5]. These papers represented a leap forward for fetal medicine in India, and are probably the first papers on these topics in India. I remember her most, however, for her charming manners, gentle voice and disarming smile, which won her many friends and patients.

The field of fetal medicine evolved in India through pioneering research by her group in the Department of Obstetrics and Gynecology, All India Institute of Medical Sciences, New Delhi along with the Genetic center at AIIMS set up by the author, with later contributions by Gogate, Chakravarti and Purandare's group in Mumbai, Suresh's group in Chennai, and other leading obstetricians scattered across India. Currently, amniocentesis is being performed in many places, and chorionic villus sampling in

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somewhat fewer centers. Ultrasound studies for determining the well-being of the fetus are available widely and numerous ultrasonologists in different cities have a high level of expertise. Biochemical screening started slowly and was then given a big push by the commercial companies which established their collecting centers all over India. Diagnosis of aneuplodies has become a major concern of obstetricians all over the country. I wish they would adopt, with equal gusto, carrier screening for thalassemia in every pregnant woman.

Screening for fetal anomalies during pregnancy is an essential part of obstetrical care, so as to reduce the burden of chromosomal diseases. This has been the recommendation of obstetric societies all over the world [6]. Table 1 compares the burden of chromosomal disorders in India to other genetic disorders. It is apparent that the burden of chromosomal disorders is second only to that due to congenital anomalies. Chromosomal diseases are associated with malformations and intellectual disability, and such individuals are dependent on the parents. About 34,000 infants with Down syndrome are born every year. This represents the highest number of infants with Down syndrome born in any country. It is a considerable burden and attempts to reduce it are desirable.

Table 1 Burden of genetic disorders in India

Disorder	Incidence	Births/Year
Congenital malformations	1:50	678,000
Chromosomal diseases	1:166	160,000
Down syndrome	1:800	34,000
Trisomy 13	1:6,500	4,100
Trisomy 15	1:12,500	2,136
β-thalassemia + SCD	1:2,700	16,700

Adapted from Ref. [7]

Conventional options to identify fetuses with trisomy 21 can be divided into screening and diagnostic techniques. Screening tests are usually based on serum biochemical tests (e.g. assay of PAPP-A, free β-HCG, α-fetoprotein) along with ultrasound scans. They are noninvasive, cheap, but are less accurate, have low detection rate, and high false positive rate. On the other hand, diagnostic tests consist of chorionic villus sampling (CVS)/amniocentesis followed by FISH or QF-PCR studies with karyotyping. CVS and amniocentesis are invasive and expensive, but are highly accurate with high detection rate and low false positive rate. The detection rate for Down syndrome using various screening strategies is set out in Table 2.

Features of both screening and diagnostic tests mentioned above, are combined in noninvasive prenatal testing (NIPT). It is based on the fact that cell-free fetal DNA (cff-DNA) is present in the maternal blood, first demonstrated by Dennis Lo et al. [8]. It is released into the maternal blood through apoptosis of cytotrophoblastic cells of placenta, as small DNA fragments (150–200 bp). It is not derived from the fetus, and this can lead to errors in diagnosis due to the presence of placental mosaicism, which get reflected in the test results. Maternal blood contains a mixture of both maternal and fetal cf-DNA, and only 5–10 % of total DNA is fetal (ranging from 2 to 20 %). The fetal DNA is reliably detected after 7+ weeks of gestation, and is undetectable within hours postpartum. This has the advantage that the test is valid from early to late pregnancy, and importantly, the DNA fragments represent only the current pregnancy.

The technologies for analyzing cff-DNA for aneuploidies are mainly of three types [9]—counting after massively parallel sequencing [Sequenom (MaterniT21), Verinata (Verifi), BGI (Nifty)] or targeted sequencing of selected chromosomes [Ariosa (Harmony T)] or single nucleotide polymorphism (SNP) analysis [Natera]. Different investigators and scientific bodies have used different terms to define this technology: NIPT, noninvasive prenatal

Table 2 Detection rate for Down syndrome using different screening techniques

Technique	Detection rate (%)
First trimester (tri)	
Nuchal translucency (NT) measurement	64–70
NT + Biochemical screening (PAPP-A + f β-HCG)	82–87
Second trimester	
Triple screen	69
Quadruple screen	81
First and second trimester combined	
Integrated test (first tri biochemical screen +NT + second tri blood screen)	94–96
Serum integrated (first tri + second tri biochemical screen)	85–88
Noninvasive prenatal screening (cff-DNA)	>99

diagnosis (NIPD), and noninvasive prenatal screening (NIPS). The last term is recommended by the American College of Medical Genetics to emphasize the screening nature of the test [10], although the NIPD has more common usage as a result of publicity by the testing companies. Bianchi et al. [11] has recommended the use of the word noninvasive DNA testing (NIDT), as this distinguishes it from other screening tests like analysis of serum analytes and ultrasound studies. However, all authorities agree that it can still only be classified as a screening test [12, 13]. This technology has changed irreversibly the field of screening during pregnancy. In the West, it has reduced the performance of invasive procedures by almost 60–70 %. It is necessary for all obstetricians to learn about this technology and have knowledge of the counseling issues involved, so that they can explain the implications and use the technology appropriately for their patients.

It is safe and accurate, has a high detection rate, high positive predictive value, and a low false positive rate [14, 15]. In India, it costs a little more than amniocentesis, although considerably more than biochemical-cum-ultrasound screening. Further advances in technology are expected to reduce the cost further. Garfield and Armstrong estimated that using NIPT as a second tier test would reduce invasive tests by 72 %, and risk of procedure-related miscarriage by 66 % [16].

The false positive rate for the biochemical screening tests (excluding NIPT) is 5 %, while the positive predictive value is 2–5 %. For NIPT, the false positive rate is much less, while the positive predictive value is much higher, almost 50 % or more. For confirmation, CVS or amniocentesis need to be carried out, but these are associated with fetal loss rate of 1 % for CVS, and 0.2 % for amniocentesis [17, 18]. The sensitivity and specificity of NIPT is compared with those of CVS and amniocentesis in Table 3. It is to be noted that NIPT has a negative predictive value of 99.6 % [18]. Even the gold standards (CVS or amniocentesis) are not 100 % sensitive and specific [16, 17].

NIPT has caused a shift in the paradigm of screening in the West [19]. The major companies in this field have set up collaborations in India, with an eye on the huge market, to provide this facility for Indian patients. Currently, all the companies are sending the samples abroad for analysis. It is learnt that one Indian diagnostic company is establishing the technology in India, in collaboration with its foreign partner.

Table 3 Sensitivity and specificity of various prenatal tests

Procedure	Sensitivity (%)	Specificity (%)
CVS (first trimester)	99.25	98.65
Amniocentesis (second trimester)	99.4	99.5
NIPT	99.6	99.8

It is generally agreed that NIPT should be offered only in the context of adequate pre-test and post-test counseling, as an option to women carrying singleton fetuses at high risk of having autosomal aneuploidy [10, 13]. The pre-test counseling session should emphasize the test's high negative predictive value, its low false positive rate, and the fact that (after 10 weeks) it does not depend on gestational age. Post-test counseling sessions need to emphasize that positive test results should be confirmed with an invasive procedure that obtains a fetal karyotype or chromosome microarray. NIPT provides two widely separated results. Most often it is reported to be negative, which is highly reassuring, and reduces the risk of trisomy 21 to less than 1 in 10,000, or it shows a high risk, with a positive predictive value of 50 % or more, even in low-risk settings.

The American College of Obstetricians and Gynecologists (ACOG) Committee has stated that cff-DNA appears to be the most effective screening test for aneuploidy in high-risk women, and is one option that can be used as a primary screening test in women at increased risk of aneuploidy [13]. NIPT should be an informed choice of the patient after pretest counseling. A patient with a positive test result should be referred for genetic counseling and should be offered invasive prenatal diagnosis for confirmation of test results [20]. Some companies also offer diagnosis in the presence of twins, but will of course, not differentiate which twin is affected [21].

The major limitations of NIPT [22–24] include test failures, unclear results (due to mosaicism), false positive results often due to detection of confined placental mosaicism, or due to a vanishing twin, and the need to confirm the abnormality in the presence of a positive test. Other aspects such as pain, fear, and discomfort are minimal in NIPT. Essentially, NIPT avoids the two major inadequacies of the current screening programs—fetal losses caused by invasive tests and the unwanted and often unanticipated births of handicapped children due to false negative test results or the decline of invasive testing due to a fear of miscarriage due to the procedure.

The main factor for a successful result after NIPT is the fetal fraction (FF) in the DNA extracted from maternal blood. In turn, the main determinants of FF are maternal weight and the gestational age [25, 26]. If the FF in the total DNA is less than 4 % it may lead to the test failure or give a false negative report. FF is dependent upon maternal BMI, at 60 kg weight FF was 11.7 %, while at 160 kg it was 3.9 %, due to the dilution effect from large maternal blood volume. FF is also low at gestation less than 10 weeks. Low percentages of mosaicism (10 %) may not be detectable and may lead to false negative results. Chromosomes that have low GC content, such as chromosome 13, lead to poor performance of the NIPT. Counting methods suffer more at low FF, as there is less distinction between the euploid and aneuploid distributions

at low FF. Only one of the technologies is able to establish the presence of a vanishing twin and triploidy.

Another advantage of NIPT is the ability to diagnose cytogenetic microdeletions with high degree of accuracy. Microdeletions are chromosomal deletions comprising 100 kb to several Mb in size [27]. Karyotype can only detect deletions/duplications of size $\geq$7–10 Mb. The common microdeletions tested are 22q11.2 deletion/DiGeorge syndrome (may have cardiac defects detected on ultrasound), 1p36 deletion, Angelman syndrome, Prader–Willi syndrome, and Cri-du-chat syndrome. One company screens additionally for chromosome 16 and 22, and deletions of 4p (Wolf–Hirschhorn syndrome), 8q (Langer–Gideon syndrome), and 11q (Jacobsen syndrome) [28]. The 22q microdeletion is the commonest and has the greatest clinical importance. It is the second most common cause of congenital heart disease, the second most common cause of developmental disability, and the most common cause of syndromic palate abnormalities [29]. It is more common than trisomy 18, 13, cystic fibrosis, spinal muscular atrophy, and fragile X syndrome. In younger women, it is more common than even Down syndrome [29]. It has also been shown that in cases of subchromosomal deletions, especially 22q deletion, early intervention matters. If diagnosed prenatally such babies should be delivered at a Tier III facility, because of the expertise required for managing congenital heart disease; no live vaccines should be administered as these infants are immune-deficient, and they should be given calcium to avoid seizures and cognitive impairment (hypocalcemic) [30]. This is a strong argument to test for this subchromosomal deletion in every case in addition to the five chromosomes that are usually tested.

At the Center of Medical Genetics, Sir Ganga Ram Hospital (SGRH) in Delhi, we have used NIPT technology for determining the presence of Y chromosome in maternal blood in X-linked disease, e.g., in Duchenne muscular dystrophy (DMD) and hemophilia, more as a research technology rather than for routine diagnostic use. However, it is in routine use for establishing the RhD positivity status of the fetus, in an RhD negative mother. It is also used as research tool for diagnosis of dominant single gene disorders when they are present in the father and are absent in the mother. For example, if the father has achondroplasia and the status of the fetus is to be determined. We are also carrying out research study on NIPT of thalassemia when the paternal mutation is different from the mother's mutation in the β-globin gene.

The Pre-Conception and Pre-Natal Diagnostic Techniques (PCPNDT) Act does permit sexing of the fetus in X-linked disease, such as Duchenne muscular dystrophy or hemophilia A or B. The cost–benefit analysis of NIPT of fetal sex in X-linked disease (XLD) is presented. It is estimated that about 25,000 women in India are at risk of X-linked disease every year (DMD, hemophilia, other disorders) based on the population prevalence of these disorders. For every 1,000 pregnant women at risk of XLD, NIPT of sex would reveal that approximately half would be female fetuses, so that only 50 % of the pregnancies would require invasive prenatal diagnosis. Without sexing, the cost of invasive tests in 1,000 women at cost of Rs. 22,000 per case would be Rs. 22 million. To this, we should add the cost of QF PCR for confirming the sex of the fetus (500 $\times$ 4,000 = Rs. 2 million), giving a total of Rs. 24 million. At this rate the cost of performing invasive prenatal diagnosis in 25,000 women would be Rs. 24 $\times$ 25 = Rs. 600 million. On the other hand, cost of sexing of the fetus based on fetal DNA in maternal blood would be 25,000 $\times$ Rs. 5,000 = Rs. 125 million. The cost of invasive testing calculated above would be halved 0.5 $\times$ 600 million = 300 million, so that the total cost would be only Rs. 425 million. This would mean a saving of Rs. 175 million.

Similarly, if we determine the RhD status of the fetus in an RhD negative pregnancy, we would be able to identify women who are carrying an RhD negative fetus, and would thus not require any anti-D prophylaxis. This would lead to an overall saving of anti-D antibody as well saving of the money spent on its purchase.

NIPT study for aneuploidies at SGRH has been carried out from December 2012 till date. Pre-test counseling was provided in each case. Information was given that this is not a diagnostic, but a high-efficiency screening test. Women were told that the NIPT will only check for aneuploidies of trisomy 21, 18, 13, X, and Y chromosome. Case history was reviewed to decide if patient should be offered invasive testing (IT), e.g., presence of genetic disease or recurrent pregnancy loss, or abnormalities on ultrasound examination not suggestive of aneuploidy of chromosomes. Data of the first 320 pregnant women tested by NIPT have been analyzed so far. Different vendors were used: BGI (China), Quest (Natera), Amniocore (Verinata). In each case, when the results were given to the patient, post-test counseling was provided. If NIPT result was negative, no further action was taken. If a positive result was obtained on NIPT, the confirmation of the abnormality was sought with amniocentesis or CVS, depending upon the gestation. The major indications for the test were positive second trimester biochemical screen (triple or quadruple test), first trimester screen positive (biochemical risk) with normal nuchal translucency, maternal age equal to or more than 38 years, IVF pregnancy, ultrasound findings suggesting the presence of an aneuploidy, and previous history of Down syndrome. No aneuploidy was detected in 308, of which 180 have delivered normal babies so far. Aneuploidy was detected in 12 fetuses. Confirmation was obtained in five fetuses with trisomy 21 and three with trisomy 18. Three cases reported as 45, X and 1 case of triploidy were not confirmed on invasive testing and were thus false positive.

Bianchi et al. [31] evaluated the performance of the NIPT in 1914 low-risk singleton pregnant women. The false

positive rate with NIPT versus standard screen was 0.3 % versus 3.6 % for trisomy 21, and 0.2 % versus 0.6 % for trisomy 18. Both techniques detected all cases of aneuploidy (T21–5; T18–2; T13–1). The negative predictive value was 100 % for both techniques. While the positive predictive value for trisomy 21 was NIPT–45.5 % versus standard screening–4.2 %, for trisomy 18 it was NIPT–40.0 % versus standard screening–8.3 %. However, the various societies observe that NIPT, although much more accurate than existing screening strategies, is still not a diagnostic assay [32]. The Canadian obstetric society says, "NIPT appears very promising for screening purposes, however more studies in average-risk pregnancies as well as lower test cost are required before it can replace the current maternal screening approaches" [33]. Secondly, before this technology becomes the primary screen for chromosomal abnormalities in pregnancy, it has to become cheaper.

Currently in India, NIPT should be used for high-risk women. It cannot be used for low-risk women until the cost comes down. The ideal use of NIPT would be in women who present with a positive first trimester combined test. As the samples are currently being sent out, it takes about 10–14 days for the result. Consequently, it cannot be used for women who already have gestation of 20 weeks or more, as the results would come beyond the legal limit of termination of pregnancy. This may change if the test is done in India which would reduce the cost as well the time for the result. Abnormal results have to be confirmed with an invasive test. The test should not be used for cases with recurrent miscarriages, family history of genetic disease, ultrasound study showing malformations, or increased nuchal translucency or nuchal fold thickness. These latter cases require a full karyotype or preferably a micorarray study.

In conclusion, one is amazed by the realization how fast the field of fetal medicine is expanding. The ultrasound machines are being perfected, and for those who know and keep with the advances, the diagnostic possibilities are limitless and are shifting to the first trimester. Similarly, there are matching advances in molecular biology techniques. The likely benefits for the pregnant women are immense and would lead to a great reduction in maternal and fetal mortality with a better quality of life for the babies who come into this world. We can look forward to a bright and exciting future.

Conflict of interest None.

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