Prenatal Diagnosis: Current Scenario

Dr Shubha Phadke
M D [Pediatrics], D M [Medical Genetics]
Professor & Head,
Department of Medical Genetics
Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, India, 226014

E mail: shubharaophadke@gmail.com

Introduction

Prenatal diagnosis has revolutionized the primary and secondary preventive interventions of birth defects and genetic disorders over last few decades. Prenatal diagnosis is done mainly with the objective of early detection of serious disorder or malformations in the fetus which gives an option of termination of pregnancy to the parents to avoid birth of a child with disability or life-threatening disorder. Prenatal diagnosis in some cases also helps in prenatal treatment or organizing postnatal treatment for good outcome. Termination of pregnancy within the legal limits of termination if the fetus is found to be affected with a serious disorder is an option acceptable to many families in India and worldwide. However, it is an emotionally taxing decision and can never be considered ethically right decision in true sense. These perspectives should be kept in mind by everyone involved in prenatal diagnosis.

Prenatal diagnosis has many aspects which need to be taken care of appropriately for the fruitful utilization of the technology (Table I). Increasing availability of expertise in ultrasonography and laboratory facilities in India need to be utilized appropriately conforming to the basic principles of medical genetics and genetic counseling as these are the pillars of prenatal diagnosis. High throughput sequencing is a revolution in twenty-first century. This powerful technique has immense abilities and also the power to reveal the unknown and uncertain genetic variations which may pose dilemmas to medical geneticists and fetal medicine specialists. The genomic techniques like cytogenetic microarray and whole exome sequencing (WES) need to be used appropriately based on the clinical situation and in correlation with the family history data, the genetic disorder in the family or the fetus and do not necessarily replace traditional techniques like karyotyping and Sanger sequencing in all scenarios.

Table I: Various Aspects of Prenatal Diagnosis & Related Issues

Aspect of Prenatal Diagnosis	Related Issues
Diagnosis of Proband	Clinical phenotype with possible genetic disorder, Stillbirth, Bad obstetric history, History of death of a child with possible genetic disorder, Genetic disorder in a relative, Previous pregnancy with fetal malformation or other ultrasonographic abnormalities.
Fetal Imaging	Ultrasonography, Fetal MRI, Post termination CT scan/ radiograph, Post termination whole body MRI [if the family is not willing for autopsy] Post termination imaging should be done as early as possible to avoid distortions due to transport of fetus in a container and formalin induced artifacts
Fetal Sampling	Chorionic villus sampling, Amniocentesis, Fetal blood sampling, Post termination cord blood / piece of cord or placental tissue
Laboratory Techniques	Traditional karyotyping , MLPA, QF PCR, Sanger sequencing, Cytogenetic microarray, Next Generation Sequencing (NGS)
Genetic Counseling	Pre –pregnancy counseling, After diagnosis of the proband, Pre test counseling, Post test counseling, Counseling for for population based screening

Indications

The situations of prenatal diagnosis can be divided into two scenarios with totally different perspectives of the families and this has to be kept in mind while talking to the family. Table II lists some of the issues in these situations. The perspective of families with previous experience of the disorders is different from the families with no family history of genetic disorder.

Table II: Psychological and Counseling Complexities in Specific Scenarios

Previous child or family member with birth defect/ genetic disorder	**Population based screening / No family history of genetic disorder**	
	USG based prenatal diagnosis	**High risk on screening test**
Risk of recurrence needs to be told before offering prenatal diagnosis	Not prepared for any abnormality Background possibilities may be conveyed without creating undue anxiety	Many people find it difficult to understand the difference between the results of a screening test & a diagnostic test
The family is aware of the possibility of recurrence	The prediction of definite prognosis may be difficult	Unless counseled before the screening test, the family may not be ready for the invasive test
The family is usually aware of the phenotype / natural course of the disorder	It may be difficult to get idea about outcome and estimate the burden of the disorder	The cost-effectiveness of the screening test should be assessed as it is offered to each pregnant woman

Previous child or family member with a genetic disease or a malformation known as the proband, is an important indication for prenatal diagnosis and accurate etiological diagnosis of the affected child / proband is the prerequisite for providing accurate prenatal diagnosis [1]. The proband can be a fetus in the previous pregnancy of mother, a stillborn, neonate who died or a child who is labeled as cerebral palsy or birth asphyxia and has not been investigated. Genetic disorders can affect any system of the body and may have presentations similar to non-genetic disorders. Sometimes the affected individual may be in the extended family like uncle, nephew, niece, etc. Such families if referred during pregnancy, the proband may not be available for evaluation or the time may be inadequate for testing of the proband and timely prenatal diagnosis may not be possible for lack of the etiological diagnosis of the proband. Table III lists the important situations which indicate possibility of genetic diseases and increased recurrence risks.

Table III: Clinical Situations Requiring Evaluation for Genetic Etiologies with the Objective for Providing Risk of Recurrence and Prenatal Diagnosis

Clinical situation	Evaluation	Remark
1. A child or an adult with a genetic disorder or a phenotype suggestive of genetic disorder	Evaluation by a pediatrician / physician or a clinical geneticist for etiological diagnosis.	Investigations will vary according to presentation and include imaging, biochemical, hematological testing, enzyme assays, histopathology but should include mutation testing for monogenic disorders or karyotype, cytogenetic microarray or MLPA for chromosomal disorders
2. Stillbirth	Though there is some evidence of neonatal respiratory problem each stillbirth to be subjected to autopsy, including photograph, radiograph, placental evaluation.	Two millilitre blood in EDTA be stored for genetic testing as per the autopsy findings. A piece of umbilical cord can be stored in normal saline at 4^0 centigrade.
3. Pregnancy terminated for prenatal diagnosis of a malformation or USG detected abnormalities	Each fetus should be subjected to fetal autopsy. Prenatal genetic testing by amniocentesis is advisable. Or postnataly blood or a piece of cord should be stored for DNA studies	Various studies have shown that the autopsy may add some malformations and change etiological diagnosis and risk of recurrence [3, 4]
4. Spontaneous abortion of a fetus of gestation more than 14 weeks or intrauterine death at any gestation	Fetal autopsy and genetic testing is essential	For genetic testing of fetal tissue, blood, a piece of cord or placenta is preferable and DNA based testing using MLPA with subtelomeric probe set is preferable as

		traditional karyotyping is not successful in many cases due to contamination or poor quality of sample
5. Three spontaneous abortions	Chromosomal analysis of products of conception at third spontaneous abortion may be done by traditional karyotyping or MLPA with subtelomeric probe set or using cytogenetic microarray	More important is karyotypes of the partners from blood. Karyotype of each spontaneous abortion is not indicated unless it is recurrent

These situations need to be evaluated prior to planning further pregnancies so that prenatal diagnosis can be facilitated. In the era of genomic testing the diagnostic yields of phenotypes suggestive of genetic disorders has increased and thus is the possibility to provide prenatal diagnosis to the family in concern. It should be noted that evaluation of the proband can not be replaced by testing the parents. If the baby is stillborn or there is a dying infant, storage of two millilitre of blood in EDTA vial or a one centimetre piece of umbilical cord will go a long way in finding out the genetic defect, mutation in the proband, which is a must for providing reliable prenatal diagnosis. Various studies have proved diagnostic utility for fetal autopsy [2]

Genetic Testing

The various forms of genetic investigations along with their advantages and limitations are given in table IV. The information is useful for appropriate utilization of these test methodologies in specific clinical scenarios. With advent of molecular cytogenetics techniques namely fluorescence in situ hybridization (FISH), MLPA and QF PCR, very minute chromosomal abnormalities could be detected. Cytogenetic microarray (CMA) has replaced traditional karyotyping due to its ability to analyze all chromosomes at very high resolution. Next generation sequencing (NGS) has revolutionized diagnosis of monogenic disorders as sequencing of coding regions of all genes (whole exome sequencing – WES) or of clinically relevant genes (Clinical Exome Sequencing) is available at affordable costs. As mentioned above these techniques play an important role in evaluation of proband with genetic disorder which is the prerequisite for the genetic counseling and prenatal diagnosis. In addition these genomic techniques play an important role in the evaluation of fetus by prenatal sampling. The role of CMA and WES or clinical exome sequencing can better be understood if the principles of techniques are understood (5, 6). In near future whole genome sequencing as a single test to identify all types of genetic variations and diagnosis of all genetic diseases will be the only and the first test. Till then one has to judiciously order the genetic test in different clinical scenarios. The information in table IV will be useful for ordering the appropriate test.

Table IV: Advantages and limitations of various cytogenetic & molecular techniques

Technique	Advantages	Indications	Limitations
Traditional karyotyping	Available easily Evaluates all chromosomes in one go	Previous child with chromosomal abnormality Increased risk of trisomy 21 One of the parents with balanced chromosomal rearrangement As an adjunct to any prenatal testing; also serves as a source of sample by way of cultured cells	Low resolution Needs live cell Reporting time of 2 to 3 weeks
QF PCR	Quick reporting (3 to 10 days) Also checks for maternal cell contamination	Increased risk of trisomy 21 or common aneuploidies	Does not detect aneuploidies of other chromosomes & structural abnormalities of all chromosomes including of chromosome 21, 13, and 18
MLPA with subtelomeric probes	Quick reporting (3 to 10 days) Useful in detecting submicroscopic microdeletions / duplications involving ends of the chromosomes Low cost as compared to CMA	Products of conception or fetal demise; less costly than CMA but can detect double segment chromosomal rearrangements which are important due to high risk of recurrence Also part of amniotic fluid can be subjected to MLPA if CMA is not available due to cost constraints	A good substitute to CMA being less costly but it will not detect many submicroscopic losses or gains detectable by CMA
MLPA for common microdeletion/ microduplication syndromes	Can detect common microdeletion syndromes like 22q21, 4p-, 5p-, etc. which are not detectable by traditional karyotyping	If fetus has cardiac or other malformations and CMA is not possible due to cost constraints	Is targeted to common regions of chromosomes while CMA can detect minute subchromosomal abnormalities anywhere in the genome
Cytogenetic Microarray	Can detect minute chromosomal abnormalities (known	Should be offered in each case where fetal sampling is done for	In about 1% of cases some CNVs detected may have uncertain

	as copy number variations -CNVs) anywhere in the genome Very high resolution chromosomal analysis as compared to karyotype	any indication Will detect clinically significant CNVs in 1% of fetal samples without ultrasonographic abnormalities Also is the first tier test for all cases with sonographic abnormalities	significance
Sanger sequencing	It is used to identify small deletions / duplications and point mutations which are the most important causes of monogenic disorders*	Prenatal diagnosis for monogenic disorders The mutation to be tested in fetal samples has to be identified in the family by testing the proband or carrier parents**	*Can not detect large deletions which are usually seen in patients with Duchenne muscular dystrophy, spinal muscular atrophy or large rearrangements like inversion in Hemophilia A, Fragile X syndrome
NGS based exome sequencing (WES)	NGS can sequence all genes or many genes in one go and hence cost and labour effective as Sanger sequencing for many genes may take a long time and will be costly	Indicated in diagnosis of the proband with a disease known to be caused by a very large gene or the causative genes can be many (e.g. retinitis pigmentosa, myopathies) or a phenotype which can not give any clue to the causative gene (e. g. Intellectual disability) If proband is not alive and the disorder in concern is likely to be autosomal or X linked recessive, the parents can be tested for carrier status of the disorder in concern	At present it is not completely effective in detecting large deletions / duplications in gene and triplet repeat disorders and CNVs The DNA sequence variations detected may be variations of uncertain significance (VUS) and good correlation with patient's phenotype and testing of family members is necessary It may detect carrier status for mutations in other genes unrelated to the phenotype and counseling regarding the significance of these variations and risk of occurrence of that disease in the baby will be needed

Note: **It should be noted that in a child with thalassemia major, there can be any one or two mutations of more than 350 reported till date. Hence pre-pregnancy testing of the proband or carrier parents for identification of mutations is essential. If it is not done before pregnancy, the mutation testing preferably should be done before obtaining fetal sample. This is true for most of the monogenic disorders as hundreds of mutations are known in each disease causing genes. Very few diseases like achondroplasia and progeria are known to be caused by only one mutation.

CMA and NGS based investigating tests namely, WES, WGS and panels including clinical exome sequencing are the techniques which can interrogate /evaluate whole genome in one go have increased the diagnostic yield and changed the approach of genetic testing. These are costly investigations, interpretation involves correlation with clinical findings and the ordering clinician needs to be aware of the possibilities of detection of novel likely pathogenic variations and variations of uncertain significance and be able to communicate these to the patient / family and provide genetic counseling. These two techniques are briefly discussed below.

Cytogenetic Microarray: CMA is based on the principle of comparative genomic hybridization. It evaluates all the chromosomes and can detect microdeletions or duplications of very small sizes. If a microarray chip with only a few regions of the genome is used, it defeats the purpose of microarray's ability of identifying abnormality of any part of any chromosome. Such CMA tests available at low cost have diagnostic limitations. CMA is offered as the first tier test for evaluation of children with intellectual disability and /or multiple malformations. CMA is also advocated also for all prenatal samples (7). For fetuses with malformations, CMA should be done even if the family has decided to terminate the pregnancy based on ultrasonographic findings. It should be noted that CMA is not suitable where the phenotype is classical of a monogenic disorder as its level of resolution is few thousand kilobases and can not detect mutations in genes.

Next Generation Sequencing: NGS is a high throughput technique for sequencing and provides sequence data of whole genome, whole exome (coding regions of all genes), clinical exome (coding regions of selected genes of clinical relevance) or phenotype specific panels (e. g. epidermolysis bullosa panel, Noonan syndrome & Rasopathy panel) (8). It provides nucleotide by nucleotide sequence of each like Sanger sequencing but on large size. For diseases like thalassemia major the causative gene is only one and the size of gene is small, Sanger sequencing is the test of choice. On the other hand if the clinical diagnosis is epidermolysis bullosa which can be caused by a number of genes, NGS based panel for epidrmolysis bullosa or clinical exome need to be ordered. At present the long reporting time for NGS based testing makes its use in prenatal diagnosis difficult. However, for fetuses with anomalies even if termination is planned WES should be done and it provides good diagnostic yield and helps in genetic counseling (9, 10). In fetuses without any ultrasonographic abnormalities or clinical suspicion due to family history; use of NGS in prenatal diagnosis has limitations and may present dilemmas if novel variations which could be pathogenic are identified. Hence, the preferred use of NGS based testing is for the diagnosis of the proband and if the proband is not available then the parents can be tested by carrier testing, preferably pre–pregnancy or during early pregnancy (11). Large gene deletions and duplications as well as copy number variations cannot be detected at present by NGS. In spite of NGS and CMA more than 50% cases remain undiagnosed. The possibility should be communicated to the families while ordering these costly tests. Another important point to stress is that prenatal diagnosis and termination of pregnancy based on novel or uncertain genetic variations without adequate evidence of pathogenicity should not be done. Pre test and post test counseling is essential component of genetic testing. While NGS is a

powerful tool for prenatal diagnosis, it is important that clinicians understand the ethical implications and parental perceptions of this testing modality (12). Soon WGS will be the single test to detect point mutations, small and large deletions / duplications and triplet repeat disorders.

Other two technological marvels, free fetal DNA (ffDNA) in maternal plasma and pre-implantation diagnosis are discussed below.

Non Invasive Prenatal Diagnosis (NIPT)

The most useful test on ffDNA in maternal plasma is fetal Rh typing for fetuses at risk due to Rh isoimmunization. It has also been used for diagnosis of monogenic disorders like thanatophoric dysplasia, beta thalassemia, etc. The use of ffDNA in maternal plasma has been shown to detect more than 99% fetuses with trisomy 21 but due to small rates of false positivity and false negativity; this test known as Non Invasive Prenatal Screening (NIPS) for common aneuploidies continues to remain a screening test. Confirmation of aneuploidy by invasive testing on fetus is essential before termination of pregnancy. Additional testing of 5 -10 microdeletion syndromes in NIPS panel is not of much use because they are too rare to be included in population based screening and are associated with very low positive predictive values.

Antenatal Screening and Non Invasive Prenatal Screening (NIPS): Antenatal screening for trisomy 21 which is the commonest cause of intellectual disability has become a part of routine care of pregnancy. However due to availability of multiple screening tests of variable detection rates and costs and lack of guidelines for India, some confusion surrounds the issue. Second trimester screening by quadruple test around 16 to 17 weeks provide good detection rates for trisomy 21 and neural tube defects (which has incidence of 5 times that of trisomy 21) along with the ultrasonographic detectable malformations at a cost effective rates for a population based screening strategy and that too within legal timeframe of terminations. NIPS is at present too costly to be used for population based screening as about Rs 100 million will be required to identify 10 fetuses with trisomy 21. The important advantage of NIPS is that it decreases the need of invasive testing by amniocentesis due to its high negative predictive value. Hence, NIPS has important role in precious pregnancies like those with assisted reproduction or history of recurrent abortions. Before offering NIPS, information about the cost and limitations (including the failure rate) should be provided (13, 14). It need to be remembered and communicated to the family that NIPS can screen only for trisomy 21, 13 and 18 and does not detect many other genetic causes of intellectual disability. With limited utility and the screening nature of NIPS it would be clearly quite wrong if worried patients would be encouraged to undertake a test that is screening for only one common disorder and is more expensive. Wide use of NIPS instead of quadruple test can lead to missing fetuses with neural tube defects and that is not justified (15).

It is important not to forget about many other genetic disorders with significant disabilities and poor outcomes. These disorders can be prevented by taking family history during first antenatal visit and use of CMA on antenatal samples. With improving training

facilities for the individuals with intellectual disabilities; these special individuals have ample opportunities to integrate with the society and lead fulfilling happy life. And hence, it should be remembered that the screening for trisomy 21 is an option and not compulsion. Same is true about termination of pregnancy with fetal abnormalities.

Preimplantaion diagnosis: For some families termination of pregnancy is not acceptable for personal or religious reasons. For them preimplantation diagnosis is an option. This needs in vitro fertilization (IVF) and testing each embryo by taking one cell from it. The embryos without the disease causing mutation are implanted. This is a well accepted and reliable technique, but the success rate is about 30 to 40%. In autosomal recessive disorders, the possibility of fetus unaffected with the disease is 75% thus 25% pregnancies undergoing prenatal diagnosis will need termination of pregnancy. So pre-implantation diagnosis has lower rate of outcome of normal baby and in addition pre-implantation diagnosis subjects the pregnancy to some increased risks associated with IVF. These include the increased risk of prematurity, growth retardation, birth defects. These needs to be communicated to the family while discussing the option of pre-implantation diagnosis. Another important indication for prenatal diagnosis is recurrent spontaneous abortions or babies with chromosomal imbalances due to balanced chromosomal rearrangement in one of the spouses. Some chromosomal rearrangements have very high possibility of imbalanced gametes and in such situations the option of preimplantation diagnosis needs to be considered. Rarely, some families have repeated babies affected with disorders on prenatal diagnosis and then they are not physically and mentally fit for repeated prenatal diagnosis and thus may prefer pre-implantation diagnosis.

First trimester ultrasonography: With good resolution ultrasonography machines and expertise first trimester ultrasonography is one earliest step to detect many major malformations. Also ultrasonographic markers for chromosomal aneuploidies during first trimester have very high sensitivity. First trimester ultrasonography has very important role in assessment of gestational age, number of fetuses, etc. and is indicated in each pregnancy. Some fetuses with major malformations also get detected. However, due to time required in getting appropriate views for aneuploidy markers, except nuchal translucency there is no role of advocating it for population based screening program for a large country like India. Many pregnancies with fetal abnormalities including structural and chromosomal, get spontaneously aborted during first trimester and screening for malformations and chromosomal disorders in second trimester is the logical and cost effective strategy.

Conclusion

Technological marvels have changed evaluation of fetus and the possibilities to help the families at risk of birth of a child with serious disorders are many. The first step in this direction is to identify the families who need further evaluation by drawing a three generation pedigree. Collecting family history is the step to be taken during the first antenatal visit. Pre-pregnancy counseling is the best time to evaluate the proband, provide genetic counseling and organize further plan as per the decisions of the family in concern. This needs timely referral, preferably as soon as a child with a genetic disorder is diagnosed or a mishap like stillbirth,

prenatal diagnosis of malformation, etc. happens. For successful use of prenatal diagnosis, in addition to ultrasonography and fetal sampling techniques knowledge of genetic disorders, understanding of principle of genetic tests, human genetics and genetic counseling is essential.

Medical genetics and prenatal diagnosis is rapidly evolving. At present technically sequencing of whole genome of fetus from ffDNA in maternal plasma has been shown to be feasible and whole exome sequencing of all neonates is being done in research settings. Though technically feasible, interpretation and appropriate use in clinical settings without causing harm to the patient and family will need a lot of research on various scientific and ethical aspects.

MESSAGES

- For any clinician involved in the process of prenatal diagnosis the first step is collecting the family history by drawing a three generation pedigree.
- Evaluation of the proband (member with a genetic disorder in the family who brings the family to notice), preferably before planning pregnancy is very essential.
- Each genetic test has different ways of evaluation of different types of genetic defect and needs to be appropriately used.
- All fetuses with stillbirths, fetuses with malformations, intrauterine deaths should be subjected to radiograph, photograph and fetal autopsy along with storage of at least two milliliter of blood in EDTA vial and a small piece of umbilical cord at 4 degree centigrade temperature.
- All babies or infants who are seriously ill, storage of 1to 4 milliliter of blood in EDTA vial can be very useful for reaching etiological diagnosis by testing later and may provide an option of prevention of recurrence by prenatal diagnosis.
- All babies with major or minor malformations, short stature should be photographed with the consent of the parents. This may provide diagnosis from experts in addition to keeping the follow up records.
- All genetic testing including prenatal diagnostic tests, need pre test and post test counseling covering relevant points.
- The risk of spontaneous abortion following amniocentesis is 1 in 500 to 1 in 1000 (16).
- For population based screening for trisomy 21 (Down syndrome), quadruple test around 16 weeks is the best option as it combines screening for open neural tube defects (five times common than trisomy 21) and ultrasonography in one go.
- All screening tests should be offered after providing information about the disorder to be screened, detection rates, need for further invasive test in some cases and the most important message to follow the time line given for testing, coming for report.
- For prenatal diagnosis based on genetic tests, genetic diagnosis of the proband and in case of monogenic disorders, mutation identification in the proband or in carrier parents is essential before proceeding for prenatal diagnosis.

- For prenatal samples collected for any indication offering cytogenetic microarray for detecting clinically significant (causes of intellectual disability, autism) microdeletions / duplications is advisable.
- NGS based testing of fetuses with malformations or intrauterine deaths can yield etiology and provide an option of accurate prenatal diagnosis in next pregnancy.
- In families with previous fetal or neonatal illness / malformation and the sample of the proband being not available, screening of parents for recessive disorders by whole exome sequencing is possible and provides good diagnostic yield. Availability of clinical information of the proband helps in interpretation.
- Antenatal or pre-pregnancy for common genetic disorders namely; beta Thalassemia, spinal muscular atrophy and fragile X syndrome should be offered to all families.
- NGS based carrier screening of all couples without family history of a genetic disorder or possibly genetic disorder is debatable as for many variants in genes for serious disorders the interpretation may not be possible. The possibilities of overestimate or underestimate of pathogenic nature of the variant can cause errors in genetic counseling and prenatal diagnosis in addition to facing the dilemmas of uncertainties.
- NGS based testing of whole exome in fetuses without any anomaly is not advisable in the current times, as many scientific and ethical issues surrounding the interpretation of rare variants and detection of late onset disorders do not yet have correct answers and guidelines.
- Genetic counseling must be non-directive.
- Offering screening tests and prenatal diagnosis is clinician's responsibility but opting for test and termination of pregnancy if the fetus is affected with any disorder or malformation is optional and is the decision of the pregnant woman and the family.
- Prenatal diagnosis provides many families the option of planning pregnancy without the tension of recurrence or occurrence of a serious disorder, but in a few cases it involves the need of termination of pregnancy and hence, the issues need to be dealt with an extra sensitivity.

References

1. Phadke SR, Pahi J, Phadke RV, Pradhan S, Agarwal SS. Importance of etiologic diagnosis of hydrocephalus as illustrated by a case of Walker Warburg syndrome. Indian Pediatr. 1997 Nov;34(11):1037-8.
2. Puri RD, Kotecha U, Lall M, Dash P, Bijarnia-Mahay S, Verma IC. Is the diagnostic yield influenced by the indication for fetal autopsy? Am J Med Genet A. 2016 Aug;170(8):2119-26.
3. Phadke SR, Gupta A. Comparison of prenatal ultrasound findings and autopsy findings in fetuses terminated after prenatal diagnosis of malformations: an experience of a clinical genetics center. J Clin Ultrasound. 2010 Jun;38(5):244-9
4. Nayak SS, Shukla A, Lewis L, Kadavigere R, Mathew M, et al. Clinical utility of fetal autopsy and its impact on genetic counseling. Prenat Diagn. 2015 Jul;35(7):685-91.

5. Rosenfeld JA, Patel A. Chromosomal Microarrays: Understanding Genetics of Neurodevelopmental Disorders and Congenital Anomalies. J Pediatr Genet. 2017 Mar;6(1):42-50.
6. Best S, Wou K, Vora N, Van der Veyver IB, Wapner R, Chitty LS. Promises, pitfalls and practicalities of prenatal whole exome sequencing. Prenat Diagn.2018 Jan;38(1):10-19.
7. American College of Obstetricians and Gynecologists Committee on Genetics. Committee Opinion No. 581: the use of chromosomal microarray analysis in prenatal diagnosis. *Obstet Gynecol* 2013; *122*(6):1374-7.
8. Stuurman KE, Joosten M, van der Burgt I, Elting M, Yntema HG, Meijers-Heijboer H, Rinne T. Prenatal ultrasound findings of rasopathies in a cohort of 424 fetuses: update on genetic testing in the NGS era. J Med Genet. 2019 Apr 30. pii:jmedgenet-2018-105746
9. Vora NL, Hui L. Next-generation sequencing and prenatal 'omics: advanced diagnostics and new insights into human development. Genet Med. 2018 Aug;20(8):791-799.
10. Petrovski S, Aggarwal V, Giordano JL, Stosic M, et al. Whole-exome sequencing in the evaluation of fetal structural anomalies: a prospective cohort study. Lancet. 2019 Feb 23;393(10173):758-767.
11. Stals KL, Wakeling M, Baptista J, Caswell R, et al. Diagnosis of lethal or prenatal-onset autosomal recessive disorders by parental exome sequencing. Prenat Diagn. 2018 Jan;38(1):33-43
12. Harris S, Gilmore K, Hardisty E, Lyerly AD, Vora NL. Ethical and counseling challenges in prenatal exome sequencing. Prenat Diagn. 2018 Nov;38(12):897-903.
13. Norton ME, Rose NC, Benn P. Noninvasive prenatal testing for fetal aneuploidy: clinical assessment and a plea for restraint. *Obstet Gynecol* 2013; *121*(4): 847-50.
14. Adams MC, Berg JS, Pearlman MD, Vora NL. Look before you leap: genomic screening in obstetrics and gynecology. Obstet Gynecol. 2015 Jun;125(6):1299-305.
15. Racusin DA, Villarreal S, Antony KM, Harris RA, et al. Role of Maternal Serum Alpha-Fetoprotein and Ultrasonography in Contemporary Detection of Spina Bifida. *Am J Perinatol* 2015; 32(14):1287-91.
16. Akolekar R, Beta J, Picciarelli G, Ogilvie C, D'Antonio F. Procedure-related risk of miscarriage following amniocentesis and chorionic villus sampling: a systematic review and meta-analysis. *Ultrasound Obstet Gynecol* 2015; *45*(1):16-26.