AMERICAN JOURNAL OF PREVENTIVE MEDICINE AND PUBLIC HEALTH, 2017
VOL 1, NO. 1:42-51
10.5455/ajpmph.281744

eJManager

REVIEW ARTICLE

Open Access

Prenatal and preimplantation genetic diagnosis - a step in right path

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ABSTRACT

Prenatal diagnosis is the procedure of diagnosing fetal abnormalities or genetic disorders, as well offer expecting parents with information and the chance to alter pregnancy management and postnatal care. Preimplantation diagnosis is a method used prior to recognize genetic defects within embryos fertilised through in vitro fertilization to avoid some diseases or disorders from being passed on the child. There are a variety of genetic techniques which plays a major role in prenatal and pre implantation genetic diagnosis. This review paper explains the role of genetic techniques in both prenatal and pre implantation diagnosis along with other available noninvasive and invasive techniques.

ARTICLE HISTORY

Received 28 August 2016
Accepted 19 October 2017
Published 23 October 2017

KEYWORDS

prenatal diagnosis; pre implantation diagnosis; genetic techniques

Introduction

Structural or functional abnormalities, including metabolic disorders can be defined as birth defects/ congenital disorder, which are present from birth [1]. As per birth defect report by March of Dimes (MOD) [2], worldwide nearly eight million births with severe birth defects per annum and ninety four percent of these births arise within countries of middle and low income. Birth abnormalities prevalence in live births is higher in India.

Major birth defects include congenital heart defects,central nervous system anomalies, Down syndrome, hemoglobinopathies, glucose-6-phosphate dehydrogenase deficiency and chromosomal imbalances [3]. It has been estimated that 70% of birth defects are preventable [1].

Prenatal Diagnosis

Many birth defects and genetic disorders can be detected early pregnancy with the help of noninvasive and invasive techniques available in this diagnosis. It is also known as antenatal diagnosis, is a technique used to diagnose the health status of an unborn fetus.

Indications of prenatal diagnosis

There are certain warnings for prenatal diagnosis such as,

- Over 35 years of maternal age [4]
- Identified or assumed family history of genetic disease [5]
- Ethnicity at high risk for genetic disease [6,7, 8]
- Multiple pregnancy losses[9]
- Teratogen [10]
- Alterations of standard ultrasound findings [11,12]
- Unusual maternal serum screen results [13,14].

Noninvasive techniques

The techniques which are not harming to both unborn fetus and mother is non invasive and some of them are

Ultrasound

Ultrasound is one of the best primary imaging noninvasive technique which helps in major detection of aneuploidies and fetal abnormalities [15]. There are 3-dimensional (3D) ultrasound (US) and 4D ultrasound complementary to two dimension ultrasound in assessing fetal abnormalities. 3D and 4D ultrasound improves the diagnostic capability by

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providing additional diagnostic information in evaluating fetal malformations, mainly in displaying cranium and face malformation, spine and extremities malformation, and abnormal physical structure of the fetus [16,17]. The malformations detected in various organs has been reported during the first twelve weeks using ultrasonography such as congenital malformations[18], central nervous system malformations[19] , some renal or neck anomalies [20] , spina bifida [21], limbs[22], congenital heart defects[23], metabolic disorders[24].

Echocardiography of fetus can be done between 18 and 22 weeks of gestational age as it gives enhanced fetal cardiac anatomy [25]. Some abnormalities may be noted starting from the first trimester of pregnancy, occasionally with the help of the transvaginal probe, particularly when an thickness of nuchal translucency increased is detected during the screening for chromosomal abnormalities [26, 27] conducted between the 11th + 6 days and the 14th week of gestation. The use of the color doppler is of greatest importance in the early echocardiography as it helps in the detection of the large vessels.

MRI

Magnetic resonance imaging (MRI) is used as an aid to ultrasound in prenatal imaging, the later become approved technique in obstetrical medicine [28]. MRI is available for fetus as alternative technique that makes it possible to find out anomalies if it is uncertain in ultrasonographic findings and to identify associated anomalies that may be undetected in USG. However, MRI is insufficient to detect skeletal limb and cardiac anomalies when compared to USG. MRI is not used regularly during pregnancy, but it is used when nonionizing imaging methods are needed or ionizing radiation is required during pregnancy [29]. Usually, it is not advisable during the first trimester.

It is accepted that MRI is not invasive to the baby growing in placenta, although the biological risk of MRI usage is not known [30]. MRI imaging is better to USG in the identification of complex anomalies such as bilateral renal agenesis, corpus callosum dysgenesis, posterior fossa malformations in third trimester, diaphragmatic hernia, NTD and lung maturation assessment [31]. It gives significant details for prenatal diagnosis, enhances diagnostic accuracy, and if abnormality is detected, disturbs the prenatal treatment, prenatal interventions and birth plan [32].

Maternal Screening Test

Maternal serum analyte (quad) screening can be done at 15 to 20 weeks of pregnancy [33, 34, 35]. This screening test measures circulating levels of four biomarkers in the maternal blood: serum alpha-fetoprotein, â-hCG (beta-human chorionic gonadotrophin), unconjugated estriol and inhibin-A [36,37]. According to these results, along with a few other minor variables (such as body mass index and pregnancy with single or twin fetus, maternal age), the patient can be adviced about risk possibility that she may be carrying an aneuploid fetus [38, 39]. Such testing has an accuracy for twins although the detection rate is lower [40], but not for triplets or other higher-order multiple pregnancies.

FTRA (first trimester risk assessment) can be added as quadruple to the above test [41,42] which is usually done at elevan to fourteen weeks of gestation or,exactly at a crown-rump length of fourty five to eighty four millimetre [43]. It involves two elements namely the ultrasound nuchal translucency, and biochemical testing for two analytes (â-hCG, PAPP-A (pregnancy-associated plasma protein-A) in mother's blood, both of which are neccesary to accurately interpret the test. Hence this quadruple test is also known as biochemical analysis of prenatal diagnosis.

Invasive techniques

Fetal tissue sampling techniques that are invasive include the following:

- Amniocentesis
- Chorionic villus sampling (CVS)
- Percutaneous umbilical blood sampling (PUBS)
- Coelocentesis

Amniocentesis

Amniocentesis is methods of diagnosing Down Syndrome and other genetic disorders. To take fetal cells from the amniotic sac (amniocentesis), hollow thin needle is inserted in a pregnant women's abdomen with the help of ultrasonography [44]. Chromosome size and banding patterns can be examined by microscope of the fetal cells allows medical laboratories to identify and arrange each of the 23 pairs of different chromosomes including sex chromosomes, which then helps as a tool in the diagnosis for genetic diseases [45]. An extra copy of chromosome 21 in a karyotype identifies Down syndrome the most common genetic disorder is a prenatal diagnostic test usually can be done between 15th to 20th week of gestation[46]. Amniotic fluid which is removed from the amniotic

sac, that can be sent to a diagnostic laboratory and embryonic cells isolated from the amniotic fluid. No anaesthetic is needed, and a result is generally provided in about 3 to 4 weeks [47]. The risk of miscarriage is reduced to 1% when it is done by experienced obstetrician [48].

Chorionic Villus Sampling (CVS)

Chorionic Villus Sampling (CVS) test is done in the tenth to tweleveth weeks of gestation [49]. Transcervical CVS done by allowing a thin tube through a vagina of a women and villi of the cervix, and using suction to take a small sample of fetal cells [50]. CVS is not recommended for the women at age above 35. The test is done by observing cells taken from the chorionic membrane or the placenta. Anaesthetic is not required, and a test result is generally available in 2 to 3 weeks. When the test is carried out by an obstetrician experienced in the technique, the risk of miscarriage related to the test is about 2% [51]. The main disadvantage may be maternal cell contamination, mosaicism in placental and failure to obtain an adequate specimen. This may result in the need for a repeat procedure or amniocentesis [52].

Cordocentesis and Coelocentesis

Also known as PUBS (Percutaneous umbilical blood sampling), fetal blood sampling, umbilical vein sampling. This chromosome analysis test is done at in the 18th week or later of high-risk pregnancies. The technique may be used for patients such as severe oligohydramnios as amniocentesis, CVS, ultrasound are inconclusive or not achievable for these kind of patients. The risk of a miscarriage related to the test is about 3 per cent [53]. Coelocentesis is a technically simple procedure which uses extracoelomic fluid as a sample, usually for an early prenatal diagnostic technique [54].

Prenatal Diagnosis of Mitochondrial Disorder

Mitochondria are subcellular organelles contain outer and inner membrane, responsible for formation of the major portion of cellular adenosine triphosphate (ATP) by oxidative phosphorylation (OXPHOS) [55]. The human diseases, such as neurodegenerative disorders, cardiovascular disorders, neurometabolic diseases, etc are related with mitochondrial dysfunctions [56,57]. Mitochondrial disorders are the most common inborn errors of metabolism; at least 15% are caused by mitochondrial DNA (mtDNA) mutations, which occur de novo or are maternally inherited [58]. Although mitochondrial dysfunctions are considered to be a rare disorder, the current epidemiological studies states that at least 1 in 5000 individuals being affected by mitochondrial dysfunction and diseases [59]. Though the occurrence of individual mutations is much higher, affecting low number in live births [60, 61], these mutations can develop disease at a small proportion of individuals [62]. At present, there is no specific cure for mitochondrial disorders, the majority of the available treatments being directed towards relieving the symptoms [63].

The inheritance of disease causing mtDNA from heteroplasmic maternal to the offspring shows a high degree of genetic and phenotypic variation between siblings [64]. This variation can be explained well by a process known as "mitochondiral genetic bottleneck" [65]. Bottle neck is the process in which large number of mtDNA molecules is decreased. Comparison of the heteroplasmic level in offspring with those of oocytes at different stages of development has revealed that the bottleneck occurs in the early stages of oogenesis [66]. Following fertilization, heteroplasmic mtDNA mutation present in the oocyte segregates to either of the two daughter cells.

This is a random process, which generates variations in the transmission of mutation to the offspring; Thus, make it possible for an unaltered heteroplasmic female to have children, who are either unaffected or mildly to severely affected [67, 68].

The primary aim of prenatal diagnosis for mitochondrial disease is to provide an accurate assessment of the risk of the fetus developing mitochondrial disease either in utero or in childhood [69]. Where mitochondrial disease is inherited in an autosomal recessive manner, as is most often the case in childhood-onset mitochondrial disease, and the genetic changes identified are novel, then the carrier status should be confirmed in each parent with additional evidence provided from functional studies supporting pathogenicity [70]. When a mtDNA mutation is responsible, then heteroplasmy levels in blood and urine should be determined in the mother and, where possible, in maternal relatives, especially previously affected children [71]. For a minority of mtDNA mutations, there is a clear correlation between the level of heteroplasmy and disease severity, but this does differ between families and assessment of fetal risk should be made in the context of how other family members have been affected. The clinical diseases which is affected by mitochondria is given in Table 1 [72].

Table 1. Clinical diseases due to mitochondrial dysfunction.

Organ	Diseases
Brain	Ataxia, Dementia, Migrane, Myoclonous, Neuronal loss, Stroke
Eye	Optic neuropathy, ophthalmoplegia, retinopathy
Peripheralnervoussystem	Neuropathy
Blood	Persons syndrome
Liver	Hepatopathy
Pancreas	Diabetes
Heart	Cardiomyopathy, Conduction disorder, wolf hirschorn syndrome,Parkinson syndrome, white syndrome
Kidney	Fanconi syndrome, Glomerulopathy
Colon	Pseudo-obstruction
Skeletal muscle	Fatigue , myopathy, weakness
Gonads	Ovarian failure

Preimplantation Diagnosis/Screening

Preimplantation genetic diagnosis (PGD) is a substitute to prenatal diagnosis for identification of genetic diseases in couples at risk of inheriting a genetic condition to their children. Recently this diagnosis has been used to enhance clinical results in IVF cycles by detecting chromosomal aneuploidies in embryos. Human embryos are abnormal upto 40% to 60% and that accounts increases to 80% in case of women with 40 years of age or above. These condition can result in decreased implantation rates in embryos transferred during IVF technique, from 30% in women below 35 years to 6% in women above or at 40 years of age [73]. Preimplantation genetic screening is a procedure of embryo genetic testing which uses cytogenetic techniques for the purposes of de novo aneuploidy screening is known as preimplantation genetic screening [74] or PGD needs DNA sampling, genetic testing, and selected embryo transfer [75] is being suggested to enhance the effectiveness of IVF by screening embryos for aneuploidy [76]. Preimplantation biopsy of blastocysts obtained by in vitro fertilization is an invasive technique. If there is no mutation in the embryo then only implantation can be done so that many diseases/ disorders can be avoided to the next generation. All the diseases/disorders can be prevented before the implantation [77]. Diana G et al., (2015) concluded in a study that is families at risk for monogenic diseases, the Double Factor-PGD is a useful tool for selection of healthy and potential embryos for transfer along with their chromosome complement [78]. The study was cross sectional. Data were collected with a pre-tested, semi-structured self-administered questionnaire. Of the 150 attendees at the course, only 125 (83.3%) consented to filling the questionnaires, while the remaining 25 declined. A total of 125 questionnaires were administered, but only 106 were properly filled and returned giving a response rate of 84.8%. The others either returned improperly filled questionnaires or did not return the questionnaires at all.

Genetic Counselling

Genetic counseling may have a better impact on risk perception accuracy in prenatal diagnosis, though some studies observed no impact at all, or only for low-risk participants [79]. Although genetic counseling and testing can be effective for a variety of disorders [80,81,82]. Extensive research is needed to assess whether genetic counseling also effectively enhances risk perceptions for other genetic predispositions. The effect of genetic counselling can be assessed for a wide range of hereditary conditions. Genetic counselors are suggested to discuss the role of family history and complete a family history assessment to provide the essential context in which counselees can know the risk information. They must also use both numerical and verbal risk calculation to communicate personal risk information, and use visual aids when communicating numerical risk information [78]. It is suggested to undergo prenatal diagnosis if there is family history of genetic diseases or other diseases/ advanced maternal age for affordable patients to avoid inheritance to offsprings.

Impact of Genetic Techniques In Prenatal and Preimplantation Diagnosis

Once abnormal is detected by ultrasonography or maternal screen test, sample is collected from the procedures such as amniocentesis, chorionic villus sampling or by other mentioned above

procedures, the disorders can be diagnosed with genetic techniques in prenatal diagnosis. DNA can be extracted from the polar bodies which is divided from primary oocyte or from embryonic cells as one blastomere from a cleavage-stage embryo or 5 to 10 trophectoderm cells from a blastocyst-stage embryo in case of preimplantation diagnosis [83]. Molecular genetic techniques plays a vital for detecting fetal anomalies.some of the techniques which used in PGD/PGS or prenatal diagnosis are given below.

Conventional Karyotype and FISH

Conventional karyotyping has been used for decades to detect chromosomal aberrations. Conventional cytogenetics is hampered by its high cost, takes too much of time and lack of technical expertise, hence other advanced techniques which is given below is used widely for detecting anomalies in the developing fetus. FISH (Flurescent InSitu Hybridisation) assists for the prenatal and PGD of some aneuploidies and chromosomal aberrations, a process then greatly supported by the sequencing of the human genome. The FISH technique was later shown to impose major technical limitations: only a select number of chromosomes was suitable for analysis (maximum of 12 probes); interpretation was often cumbersome because hybridization failure, signal overlap, and splitting affect the accuracy of the output; and more importantly, several studies showed no difference in clinical outcomes for this technique [84, 85,86].

Biochemical Analysis

In recent years, the measurement of human chorionic gonadotrophin (HCG) and pregnancy-associated plasma protein A (PAPP-A) in maternal serum between the 11th and 14th weeks of pregnancy have become increasingly established in combination with nuchal translucency measurement and maternal age (combined first trimester test) [87]. Prior to this, the so-called triple test was offered [88], by measuring the concentration of alpha fetoprotein (AFP), HCG and free estriol between fifteen to twenty weeks.

In addition, Inhibin A,biochemical parameter when added to the triple test, results in quadruple test [89]. Exact assessment of gestational age is important for interpreting the biochemical parameters as already mentioned above briefly.

CGH

The primary component of aCGH needs labelled DNA from both test and control patients; the labelled DNA is then hybridized to a DNA microarray. By scanning and imaging the array analysis of CGH can be performed, then the intensity can be measured for test and control samples, hybridization signals relative to each probe. Finally, a computer program analyzes the data and generates a plot [90]. Initially, the study was performed with a microscope using metaphase CGH [91, 92]. For practical and accuracy reasons, metaphase CGH was easily replaced by aCGH. The assessment by aCGH determines if any quantitative deviations (extra or missing DNA sequences) is present in the DNA of the test case. Therefore it can identify chromosomal copy number (e.g., trisomies or monosomy) and unbalanced chromosome translocations [93,94]. Rearranged balanced chromosome such as inversions or translocations cannot be identified by aCGH as only genetic material is altered but no gain or loss.

qPCR

An other method for 24-chromosome copy number analysis that can be do by real-time quantitative polymerase chain reaction (qPCR) was developed and widely validated [95]. In this method, high-order multiplex PCR reaction in a 384-multiwell plate format, then a preamplification step is used to amplify at least two sequences on each arm of every chromosome. Realtime qPCR is then used for the rapid quantification of each product, allowing comparison across the genome.The multiplex PCR is executed directly on the sample to prevent amplification bias from whole-genome amplification and make sure that exact amount of copy number analysis [96].

SNP Microarray

An SNP is a DNA sequence variation in which, at a specific position or locus, one of two or more nucleotides may be present on different chromosomes within a population. To date, almost fourty million SNPs have been demonstrated across the genome mainly in non-coding regions. Most SNP arrays detect upto 2 million SNPs across the length of all chromosomes. For molecular cytogenetics, analysis of the ratio of the intensity of both alleles at heterozygous loci allows high resolution detection of duplications in, and deletions from, whole chromosomes in small regions. In deletions, loss of heterozygosity is detected by the absence of the heterozygous band [97]. SNP arrays also have benefit to find out any abnormalities of parental origin can be investigated by genotyping the parents,

allowing the detection of uniparental disomy among others. Because SNP-based approaches provide extra theoretical resolution and parent-of-origin information, they may be particularly suited to some applications such as PGD of single gene defects or unbalance translocation of a chromosome combined with broad detection of abnormalities. In addition, SNP microarray can differentiate between balanced and normal chromosomes in embryos from a translocation carrier [98].

Chromosomal microarray analysis (CMA) is a technology used for the detection of clinically-significant microdeletions or duplications, with a high sensitivity for submicroscopic aberrations. It is capable to detect changes from 5-10Kb in size - a resolution up to thousand times higher than conventional karyotyping. CMA is used for uncovering copy number variants (CNVs) thought to play an important role in the pathogenesis of a variety of disorders, primarily neurodevelopmental disorders and congenital anomalies. CMA may be applied in the prenatal or postnatal setting, with unique benefits and limitations in each setting. The growing use of CMA makes it essential for practicing physicians to understand the principles of this technology and be aware of its powers and limitations [99].

NGS

Next-generation sequencing (NGS) is recent technique that can detect chromosome imbalances in embryos as well as aneuploidy screening on single cells. Advanced level of consistency with aCGH, NGS has been established to be a robust high-throughput technique helps in PGD for chromosomal translocations as clinical application in reproductive medicine, with advantages of automation, higher throughput and reduced cost [100, 101]. Its robustness, reliability and reproducibility helps in prenatal diagnosis. This technique could guarantee an ample and quick analysis of the genes involved in development, making it possible to organize medical interventions during pregnancy and after birth [102]. This is the first study reporting extensive preclinical validation and accuracy assessment of NGS-based comprehensive aneuploidy screening on single cells. Tan et al., (2014) did a study on 395 couples who were carriers of translocations or other kinds of mutations or recurrent miscarriage. PGD/PGS screening were done for all biopsied embryos through NGS and the outcome measures of both the NGS and SNP array cycles were the same with insignificant differences. Totally one hundred and fifty blastocysts underwent NGS analysis, of which seven blastocysts were found with contradictory signals but also other signals retreived from NGS analysis were established to be precise by validation with qPCR. NGS testing was evaluated, and a significant difference was found between chromosomally normal and the abnormal blastocysts [103]. NGS gives an exact approach to detect embryonic imbalanced segmental rearrangements, to prevent the potential risks of false signals from SNP array which helps to increase in the implantation rates during in-vitro fertilization.

Ethics

Ethical issues associated with prenatal diagnosis/PGD, including the physician's role to assist risk information about pregnancy decisions and physician contribution in genetic selection and manipulation. In general, it would be permissible ethically to involve in genetic selection (abortion or embryo discard) or genetic manipulation to prevent, cure, or treat genetic disease [104].

Conclusion

Varieties of techniques are available in prenatal diagnosis which helps to diagnose genetic disorders at minimum/no risk to both fetus and mother. Preimplantation diagnosis helps to implant healthy embryos if no mutation is detected. With the help of minimally invasive molecular genetics technique, genetic diseases can be detected. Prenatal diagnosis can be offered for affordable patients as it is a right path to prevent inborn diseases to offsprings.

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