Review Article

Fetal Interventions and Fetal Diagnostic Tests—When to Order What

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ABSTRACT

The major aim of prenatal diagnosis is prevention of mental and physical handicap. This involves both low risk women without any family history and high risk women who have a family history of a child with malformations, child with intellectual disability or autism. In low risk women fetal disorder is suspected on the basis of abnormal biochemical screen, abnormal ultrasound etc. Fetal diagnosis is mainly done by CVS/amniocentesis and genetic testing is ordered based upon clinical indication.

Key words: CVS, amniocentesis, Microarray, FISH, clinical exome sequencing.

INTRODUCTION

The primary objective of genetic tests is the exclusion of any intellectual or physical impairment in the ongoing pregnancy. Genetic test either Prenatal or Perinatal depend on individual history, pregnancy history and native cultural background.[1]

INDICATIONS OF A FETAL GENETIC TESTING

Worldwide 2-3% children are born with birth defects. The main objective of obstetric care for many years has been to identify fetal disorders during pregnancy. Women seeking prenatal diagnosis can basically be divided into two groups: High risk and low risk according to family history. High-risk women already have a previous affected child or a family member with a physical or mental disability. A low risk woman has been offered prenatal diagnosis after the results of prenatal screening tests done during pregnancy. Indications of prenatal diagnosis are shown in **Table 1**.

- Positive screening tests for aneuploidy e.g. positive combined screen or positive cell free fetal DNA.
- *Parental carrier of balanced translocation:* Person with balanced translocation carriers are themselves healthy but are liable to produce unbalanced gametes due to gain or loss of involved chromosomes.
- *Parents with aneuploidy:* A trisomy 21/Down's syndrome woman is at an increased risk of having a child with Trisomy.[2] 47, XXX and 47, XYY cases are usually reproductive and there is limited data available about risk of having children with a trisomy.[3]
- *Single gene disorder carrier couple:* If both Partners are carriers of any recessive disorder for e.g. Cystic Fibrosis, Tay Sachs disease or Thalassemia, there is a 25% chance of having an affected child. Where as in an autosomal dominant disorder for e.g. Neurofibromatosis have a 50% risk of transmission. Sometimes a new mutation is seen in a child, where both parents are normal and absent family history. In such cases molecular genetic testing of affected

Table 1

Common indications of prenatal diagnosis

Current Pregnancy
• Advanced maternal age
• Abnormal ultrasound – Soft markers on USG – Malformations on USG
• Positive biochemical screen test
• Abnormal HPLC
Previous CHID
• Previous child with Thalassemia
• Previous child with Down syndrome
• Previous child with MR
• Family history of genetic disorder
• Balanced translocation carrier

child is necessary specially for evaluating risk for future pregnancy.[4]

- *History of having a child with aneuploidy or trisomy:* There is a increased risk of recurrence in subsequent pregnancy due to parameters like maternal age, type of genetic disorder in index case.[4,5]
- *Antenatal ultrasound showing structural defects:* An antenatal scan showing structural abnormalities increases the risk of genetic disorder in fetus. First and second trimester ultrasonographic soft markers are Nuchal translucency (NT)/Nuchal fold thickness (NFT), hypoplastic nasal bone, ventriculomegaly and aberrant subclavian artery[6] etc.

If the anomaly is detected by a screening test, then:

- Further confirmation tests are required (Chorionic villous sampling (CVS)/Amniocentesis)
- Genetic counselling and modified obstetric supervision (MTP (medical termination of pregnancy)/neonatal care unit)

Invasive Diagnostic Procedure

Common methods of prenatal diagnosis are shown in **Table 2**.

Table 2
Methods of prenatal diagnosis

Invasive	*Non-Invasive*
Amniocentesis	Maternal serum alpha-feto-protein (MSAFP)
Chorionic villus sampling	Maternal serum screen
Cordocentesis	Ultrasonography
Preimplatation genetic diagnosis	Fetal MRI
Fetoscopy	NIPS, (Noninvasive prenatal screening)

CHORONIC VILLOUS SAMPLING (CVS)[7]

The ideal time to perform the CVS is between 10 and 14 weeks of gestation. With the introduction of the NIPT, the frequency of CVS performance has drastically decreased. CVS remains the only diagnostic test available in the first quarter. It allows for diagnostic analysis including in situ fluorescent hybridization (FISH), karyotype, microarrays, molecular tests and gene sequencing.

Indication of CVS is mainly for prenatal diagnosis of single gene disorders.

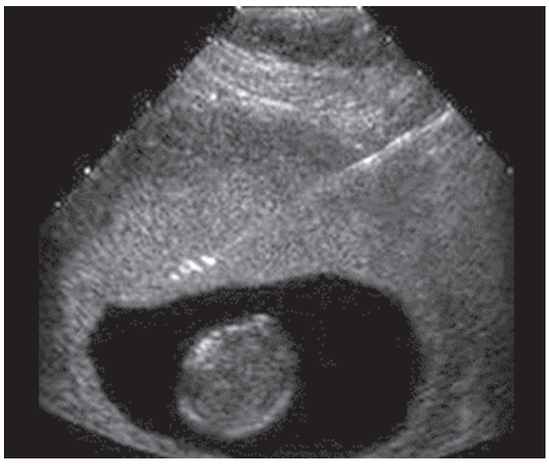

Figure 1 Chorionic villus sampling (transabdominal)—11-12 week

Procedure (Figure 1)

CVS may be performed via either transcervical or transabdominal approach. A 18 gauge spinal needle is used and by applying suction and back and forth movements in the placenta sampling is done. Ideal amount of sample is 25-30 mg of chorionic villi. This will give a backup of sample so as to avoid resampling.

Complications

- Pregnancy loss associated with CVS is reported to be 0.2-2%.
- The risk of amniotic fluid leakage following CVS is exceedingly rare—<0.5%procedures.
- Risk of chorioamnionitis very rare—1–2/3000

AMNIOCENTESIS[7]

This procedure became the first routine genetic invasive prenatal test at the end of the 1960s and is still considered the gold standard for invasive prenatal testing. Amniocentesis should be preferred method of prenatal diagnosis for chromosomal disorders and is performed at or beyond 15+0 completed weeks of gestation.

PROCEDURE (FIGURE 2)

A 20–22G needle should be inserted transabdominally under continuous ultrasound guidance. Firm entry is suggested to prevent tenting of the amniotic membrane. About 20 ml of amniotic fluid is aspirated. The first 2 mL

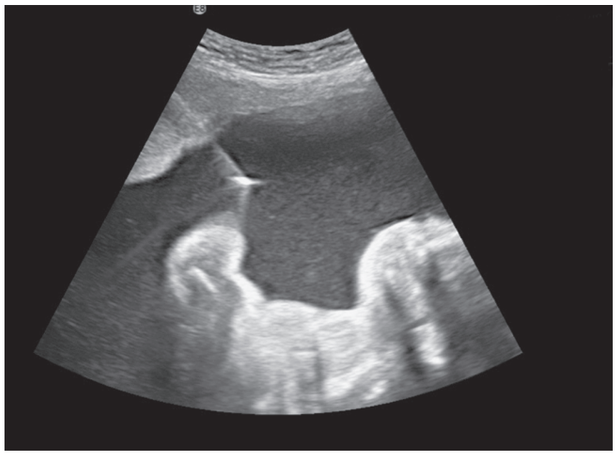

Figure 2 Amniocentesis—16-18 weeks

of fluid should be discarded to minimize contamination with maternal cells.Post-procedure avoidance of strenuous activity can be advised for about 2 days. Pre-procedure antibiotic prophylaxis is generally advised.

Complications of Amniocentesis

- Risk of fetal loss has been reported to vary from 0.1% to 1%, with recent reports being closer to the lower limit.
- The risk of membrane rupture after amniocentesis is 1–2%.

CORDOCENTESIS

Percutaneous umbilical blood sampling (PUBS), also called cordocentesis, is a technique that enables blood samples to be obtained from the fetus in utero for a variety of conditions. The major applications are for the diagnosis of fetal infections, karyotype analysis, diagnosis of hematologic conditions, and metabolic evaluation. Ideal time to perform Cordocentesis is 18 week of pregnancy and thereafter.It has highest risk of complications as compared with other diagnostic tests.

Complications

- Fetal bleeding
- Cord hematoma
- Bradycardia
- Infection
- Fetal-maternal bleeding
- Pregnancy loss

Various genetic tests and their indications are depicted in **Table 3**.

Table 3

Genetic/Genomic Testing in Prenatal Diagnosis

Basic understanding of genetic testing	
Genetic disorders	*Genetic testing*
Chromosomal Microdeletion syndrome	Karyotype FISH Microarray BACS on Beads
Single gene disorders	PCR (Polymerase chain reaction) QF PCR (Quantitative florescent PCR) MLPA (Multiplex ligation probe amplification) Exome sequencing

KARYOTYPING

Karyotyping is the gold standard for detecting fetal chromosomal aberrations. The karyotype refers to the complete set of chromosomes in an organism. **Figure 3** demonstrates a karyotype of an individual showing a normal female karyotype 46, XX.

FISH (FLOURESCENCE IN SITU HYBRIDISATION)

FISH is a technique of hybridisation of complementary DNA using fluorescent probes. FISH is commonly used for rapid diagnosis of aneuploidy of chr 13, 18, 21 and sex chromosomes. while maintaining sensitivities as high as 97.9% and specificities as high as 100%.[8]

Figure 4 demonstrates Interphase FISH showing 3 signals for chromosome 18 on amniotic fluid in a fetus with omphalocele and clubfoot.

CHOROMOSOMAL MICROARRAY ANALYSIS

Chromosomal microarray analysis (CMA) is a high-resolution whole-genome screening technique that can identify smaller submicroscopic deletions and duplications known as copy-number variants (CNVs). CMA testing **(Figure 5)** is well established in postnatal evaluation of a child with intellectual disability/autism/malformations. Diagnostic yield is up to 15% over

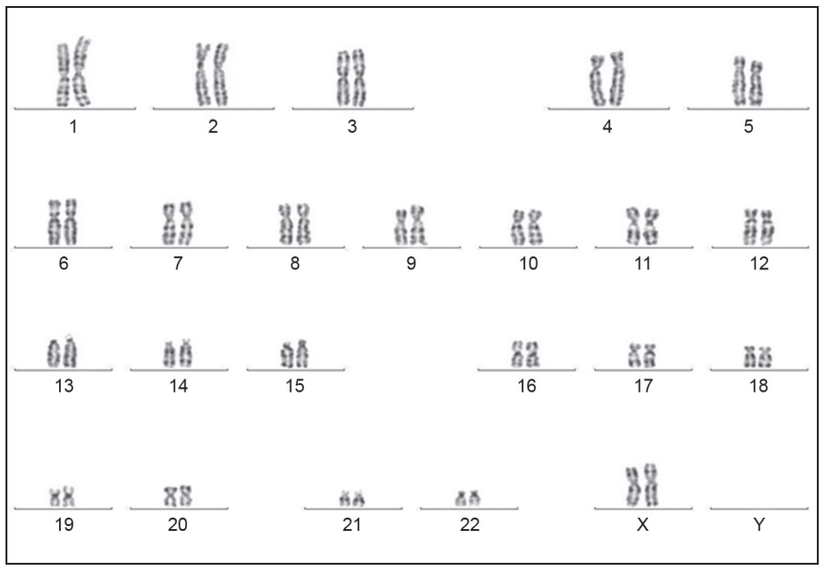

Figure 3 Normal female karyotype 46, XX

Figure 4 Interphase FISH showing 3 signals for chromosome 18

conventional karyotyping.[9,10]

In prenatal diagnosis this is indicated mainly in[11]

1. Fetus with ultrasound detected malformations
2. Increased NT>3.5 mm or NT above 95th centile

Advantage

- Higher sensitivity
- Reduced turnaround time
- Less labour-intensive work and use of standardized computerized analysis, unlike karyotyping, which uses microscopic examination of chromosomes.

QF-PCR (QUANTITATIVE FLOURESCENT PCR)

QF-PCR is a PCR analysis that produces fluorescent labelled amplicons with end point detection by capillary electrophoresis. The most commonly used method for QF-PCR involves the amplification of short tandem repetitions (STR), which are short DNA sequences specific to chromosomes, typically 2 to 5 base pairs, are stable and vary in length between subjects.[12]

Advantage

Rapid diagnosis of chromosomal aneuploidies and provide clinically valid results, reducing the workload on cytogenetic laboratories and providing faster results for patients and physicians.

WHOLE EXOME SEQUENCING (WES)

All the protein-coding genes in a genome, known as the exome, can be sequenced through WES by NGS. (Next generation sequencing). In foetuses with sonographic abnormalities, WES can provide more diagnostic potential, which would then expand the capacity to counsel families. Utility of exome sequencing in prenatal diagnosis is evolving. In general, exome

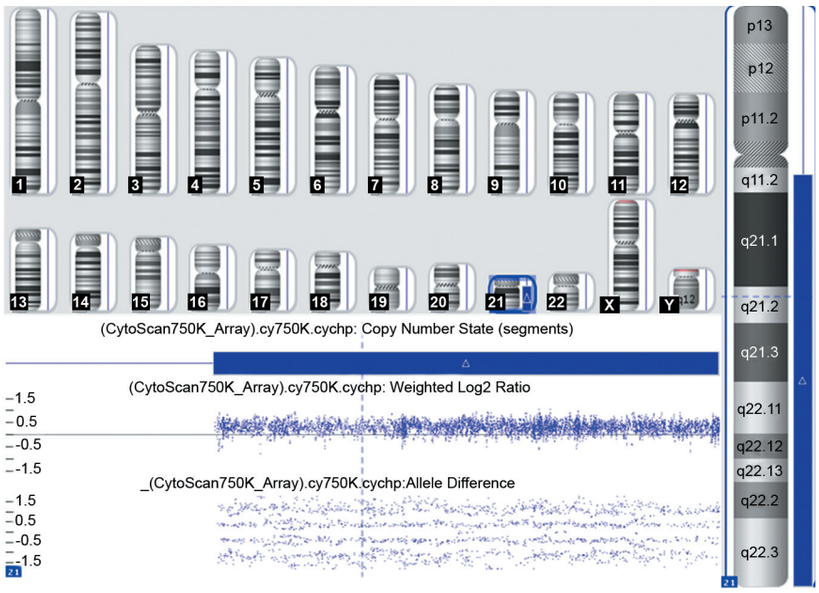

Figure 5 Chromosomal microarray showing trisomy 21 in amniotic fluid of a fetus with raised NT ad megacystis (enlarged bladder)

sequencing should be ordered when there is a history of consanguinity or family history in case with fetal malformations.

Table 4

When to order what

Anomaly	*Test*
Screen positive for aneuploidy	NIPT or FISH/Culture
Fetal anomaly	FISH + Microarray
Increased NT	FISH + Microarray and NGS for Noonan panel
IUGR	FISH + Microarray
Infections	PCR for infections e.g. TORCH
Couple Thalassemia Trait	PCR for B Thalassemia
Previous child with Mental retardation	Microarray
Previous child with mental retardation/consanguinity/ family history	Exome sequencing

CONCLUSIONS

Fetal diagnosis is important for counselling regarding prognosis of current pregnancy. This is a prerequisite in counselling for future pregnancy. Fetal screening and diagnosis can be achieved both noninvasively/ invasively. Amniocentesis and CVS are common fetal interventions. Amniocentesis is generally a preferred method for diagnosis of chromosomal disorders, wheras CVS should be the procedure of choice in molecular diagnosis.

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