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 chorionic villus sampling (CVS) amniocentesis and genetic testing is ordered based upon clinical indication.

Keywords: 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 (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.

**Table 1**

<table>
<thead>
<tr>
<th>Common indications of prenatal diagnosis</th>
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<tbody>
<tr>
<td>Current Pregnancy</td>
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<tr>
<td>- Advanced maternal age</td>
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<tr>
<td>- Abnormal ultrasound</td>
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<td>- Soft markers on USG</td>
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<td>- Malformations on USG</td>
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<tr>
<td>- Positive biochemical screen test</td>
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<td>- Abnormal HPLC</td>
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<tr>
<td>Previous Child</td>
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<tr>
<td>- Previous child with Thalassemia</td>
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<tr>
<td>- Previous child with Down syndrome</td>
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<tr>
<td>- Previous child with Mental Retardation</td>
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<tr>
<td>- Family history of genetic disorder</td>
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<tr>
<td>- Balanced translocation carrier</td>
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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 child is necessary specially for evaluating risk for future pregnancy.4

- **History of having a child with aneuploidy or trisomy:** There is an 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 artery6 etc.

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

- Further confirmation tests are required [Chorionic villus sampling (CVS)/Amniocentesis]
- Genetic counselling and modified obstetric supervisio[Medical termination of pregnancy (MTP)/NICU care (neonatal intensive unit care)].

**Invasive Diagnostic Procedure**

Common methods of prenatal diagnosis are (Table 2):

<table>
<thead>
<tr>
<th>Table 2</th>
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<tr>
<td>Methods of prenatal diagnosis</td>
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<tr>
<td><strong>Invasive</strong></td>
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<tr>
<td>Amniocentesis</td>
</tr>
<tr>
<td>Chorionic villus sampling</td>
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<tr>
<td>Cordocentesis</td>
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<tr>
<td>Preimplantation genetic diagnosis</td>
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<tr>
<td>Fetoscopy</td>
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**CHORIONIC VILLUS SAMPLING (CVS)**

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 fluorescent in situ hybridisation (FISH), karyotype, microarrays, molecular tests and gene sequencing.

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

**Procedure (Fig. 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 (0.5%).
- Risk of chorioamnionitis is very rare (1-2/3000 procedures).

**AMNIOCENTESIS**

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 (FIG. 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 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 weeks 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

<table>
<thead>
<tr>
<th>Genetic disorders</th>
<th>Genetic testing</th>
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</thead>
<tbody>
<tr>
<td>Chromosomal Microdeletion syndrome</td>
<td>Karyotype FISH Microarray BACS on Beads</td>
</tr>
<tr>
<td>Single gene disorders</td>
<td>PCR (Polymerase chain reaction) QF PCR (Quantitative fluorescent PCR) MLPA (Multiplex ligation probe amplification) Exome sequencing</td>
</tr>
</tbody>
</table>

KARYOTYPING

Karyotyping is the gold standard for detecting fetal chromosomal aberrations. The karyotype refers to the complete set of chromosomes in an organism. Karyotyping using amniotic fluid is considered the “gold standard” in fetal aneuploidy testing due to high sensitivity and relatively low risks. Figure 3 demonstrates a karyotype of an individual showing a normal female karyotype 46, XX.

FLUORESCENCE IN SITU HYBRIDIZATION (FISH)

FISH is a technique of hybridization of complementary DNA using fluorescent probes. FISH is commonly used for rapid diagnosis of aneuploidy of chr 13, 18, 21 and sex chromosomes. It has a high sensitivity (97.9%) and high specificity (100%).

Figure 4 demonstrates Interphase FISH showing 3 signals for chromosome 18 on amniotic fluid in a fetus with omphalocele and clubfoot.
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 (Fig. 5) is well established in postnatal evaluation of a child with intellectual disability/autism/malformations. Diagnostic yield is up to 15% over 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 turn around time.
- Less labor-intensive work and use of standardized computerized analysis, unlike karyotyping, which uses microscopic examination of chromosomes.

**QF-PCR (QUANTITATIVE FLUORESCENT 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 Next generation sequencing (NGS). In fetuses 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 sequencing should be ordered when there is a history of consanguinity or family history in case with fetal malformations.

Table 4 summarizes the indications of various fetal diagnostic test.

**CONCLUSION**

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, whereas CVS should be the procedure of choice in molecular diagnosis.

**Table 4**

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

**Source of Support**

Nil

**Conflict of Interest**

There are no conflict of interest.
REFERENCES