Prenatal Genetic Screening and Diagnosis

By

Dr. Inaam Faisal

Aims: Detection and identification of couples (individuals) who are at high risk for having a child with an inherited (chromosomal or genetic) disorder.

Noninvasive screening for chromosomal anomaly (trisomy 21, 18, 13) should be a routine to all pregnant women, irrespective of their age. Women who are screen positive should be offered fetal karyotyping for confirmation

Risk factors for Prenatal genetic screening

Maternal risk factors

- ☐ Maternal age > 35 years
- ☐ Family history of neural tube defects
- ☐ Previous baby born with neural tube defect
- ☐ Previous child with chromosomal anomaly
- ☐ One or both parents—carriers of sex-linked or autosomal traits
- ☐ One parent is known to carry a balanced translocation
- ☐ History of recurrent miscarriage

Prenatal risk factors

- ☐ Oligohydramnios
- ☐ Polyhydramnios
- ☐ Severe symmetrical fetal growth restriction
- ☐ Abnormal ultrasound findings (structural anomalies)
- ☐ Uncontrolled diabetes mellitus in the periconceptionalperiod
- ☐ Contact with infection (teratogenic), e.g. rubella, cytomegalovirus or intake of teratogenic drugs
- ☐ Presence of soft tissue markers of chromosomal anomaly on ultrasonography
- ☐ Abnormal maternal serum screening

BIOCHEMICAL ANALYTES

a. Maternal serum alpha fetoprotein (MSAFP): AFP is an oncofetal protein (molecular weight 70,000). It is produced by yolk sac and fetal liver. Highest level of AFP in fetal serum and amniotic fluid is reached around 13 weeks and thereafter it decreases. Maternal serum level reaches a peak around 32 weeks.

MSAFP level is *elevated* in a number of conditions: (a) wrong gestational age, (b) open neural tube defects (NTDs), (c) multiple pregnancy, Rh isoimmunization, (d) IUFD, (e) anterior abdominal wall defects and (f) renal anomalies.

Low levels are found in trisomies (Down's syndrome), gestational trophoblastic disease.

b. Inhibin A is a dimeric glycoprotein. It is produced by the corpus luteum and the placenta. Serum level of inhibin A is *raised* in women carrying a fetus with Down's syndrome.

c. Others: hCG , uE3 , PAPP

Screening Method

First Trimester Screening

Screening *parameters* are

(A) Biophysical: (i) ultrasound measurement of nuchal translucency (NT),

(ii) Nasal bone,

(B) Biochemical: (i) free β-hCG, (ii) PAPP-A (Pregnancy Associated Plasma Protein-A).

Time of Test: Between 11 weeks and 14 weeks.

Values: PAPP-A—reduced; β-hCG—increased; NT—measurement increased in trisomy 21.

NT is the fluid-filled space (detected by USG) between the fetal skin and the underlying soft tissue at the region of the fetal neck. NT ≥ 3 mm is abnormal. Combined tests can detect tristomy 21 in 92% cases with a false-positive rate of 5%.

First trimester screening is either equal or even superior to second trimester screening.

Advantages: Once a woman is screen positive, diagnostic tests should be done early.

A targeted ultrasound examination during the second trimester and fetal echocardiography are to be done when NT is ≥ 3 mm.

Second Trimester Screening

It is done between 15 weeks and 22 weeks.

MSAFP: This test is done between 15 weeks and 20 weeks. MSAFP value of 2.5 multiples of the median (MOM) when adjusted with maternal weight and ethnicity is taken as cut-off point. *Elevated* MSAFP detects 85% of all neural tube defects. Cases with such high values are considered for high resolution ultrasound imaging and/or amniocentesis. *Very low* MSAFP levels are associated with increased rates of miscarriage, stillbirth and neonatal death.

Triple Test: It is a combined biochemical test which includes MSAFP, hCG and uE3 (unconjugated estriol).
Maternal age in relation to confirmed gestation age is also taken into account. It is used for detection of Down's syndrome. In an affected pregnancy, levels of MSAFP and uE3 tend to be low while that of hCG is high. It is performed at 15–22 weeks. It gives a risk ratio and for confirmation CVS/amniocentesis has to be done. The result is considered to be screen positive if the risk ratio is 1:250 or greater.

Quadruple (Quad) Screening includes four biochemical analytes: (1) Maternal Serum Alpha Fetoprotein (MSAFP), (2) Unconjugated estriol (uE3), (3) dimeric inhibin-A and (4) hCG.

Quadscreen can detect trisomy 21 in 85% of cases with a false-positive rate of 0.9%. Levels of serum analytes in cases with trisomy 21: hCG—increased; uE3—reduced; inhibin A—elevated; MSAFP—reduced.

Adjustments are to be made for maternal age, weight and ethnic group.

Best screening procedure is combined first and second trimester procedures (ACOG).

Prenatal diagnosis

Screen positive women are offered fetal karyotyping. Fetal tissues are obtained for confirmation of diagnosis.

The procedures are: *(a) invasive and (b) noninvasive.*

Invasive Procedures for Prenatal Diagnosis:

- ☐ Chorionic villus Sampling (CVS)
- ☐ Amniocentesis
- ☐ Cordocentesis or Percutaneous Umbilical Blood Sampling (PUBS)

Noninvasive Procedures

- ☐Fetal DNA
- ☐ Biophysical

Invasive Procedures for Prenatal Diagnosis:

☐ *Chorionic Villus Sampling (CVS)* is *performed* for prenatal diagnosis of **genetic disorders.** It is *carried out* transcervically between 10 weeks and 13 weeks and transabdominally from 10 weeks to term.

Diagnosis can be obtained by 24 hours, and as such, if termination is considered, it can be done in the first trimester safely. A few villi are collected from the chorion frondosum under ultrasonic guidance. About 15–25 mg of villi are aspirated in a 20 mL syringe creating a negative pressure. The tissues are obtained in a tissue culture media within the syringe. Transabdominal (TA-CVS) is done using a spinal needle (18–20 gauge) under ultrasound guidance. it provides earlier diagnosis than amniotic fluid studies.

Complications are: fetal loss (1–2%), oromandibular limb deformities or vaginal bleeding. False-positive results (2–3%) are there due to placental mosaics and maternal cell contamination. In such a situation, amniocentesis should be performed to confirm the diagnosis. **Limb reduction deformity (LRD) is low when CVS is performed** after 9 completed weeks of gestation. CVS performed between 10 weeks and 13 weeks of gestation is safe and accurate as that of amniocentesis. Placenta biopsy has mostly replaced cordocentesis. This procedure is of low risks, technically easier and cytogenetic results are obtained within 24–48 hours.

Pregnancy termination when needed can be done a safely in the early weeks of gestation.

TC-CVS is avoided in cases with, cervical myoma, acutely angulated uterus, uterine malformations or in presence of infections, such as the genital herpes or cervicitis or in presence of vaginal bleeding.

Anti-D immunoglobulin 50 µg IM should be administered following the procedure to a Rh-negative woman. The information obtained by CVS, amniocentesis or cordocentesis is discussed below.

Structural chromosomal abnormalities (translocations, inversions, mutations) can be detected by fl uorescence in situ hybridization (FISH). Chromosome-specific probes can be used to detect the unknown DNA.

☐ *Genetic Amniocentesis* is an invaisve procedure. It is performed after 15 weeks under ultrasonographic guidance .The fetal cells obtained in this procedure are subjected for cytogenetic analysis.

Early amniocentesis has been carried out at 12–14 weeks of gestation.. Genetic amniocentesis before 13 weeks is not recommended (ACOG).

Fetal Blood Sampling

☐ *Cordocentesis (Percutaneous Umbilical Blood Sampling)*

A 22-gauge spinal needle, 13 cm in length, is inserted through the maternal abdominal and uterine wall under real-time ultrasound guidance using a curvilinear probe. The needle tip is progressed carefully and it punctures the umbilical vein approximately 1–2 cm from the placental insertion. Umbilical vein is preferred. *The advantages* are: (a) vein is larger in size (b) causes less bradycardia and (c) less hemorrhage.

Generally, 0.5–2 mL of fetal blood is collected. It is performed under local anesthetic usually from
18 weeks of gestation.

RISKS: This invasive procedure may lead to abortion, preterm labor and intrauterine fetal death. These may be due to bleeding, cord hematoma formation, infection (amnionitis), fetomaternal hemorrhage or preterm rupture of membranes. Overall fetal loss is 1–2%.

Anti-D immunoglobulin 100 µg IM should be given to Rh-negative, yet unimmunized woman.

All the information as obtained in amniocentesis or chorion villus sampling, could be gathered.

Additional values are mentioned below.

Hematological — For fetal anemia , bleeding disorders (autoimmune thrombocytopenia), Rh disease and hemoglobinopathies

Fetal infections — Toxoplasmosis, viral infections ,

Fetal blood gas and acid-base status— In fetal growth restriction

Fetal therapy — Blood transfusion, drug therapy

Prenatal diagnosis: CVs, amniocentesis and Cordocentesis

	Chorionic Villus sampling	Amniocentesis	Cordocentesis
time	Transcervical 10–13 weeks, Transabdominal 10 weeks to term	After 15 weeks (early12–14 weeks)	18–20 weeks
Materials for study	Trophoblast cells	☐ Fetal broblast ☐ Fluid for biochemical study	☐ Fetal white blood cells and) (others :infection biochemical study
Karyotype result	☐ Direct preparation: 24–48 hours. ☐ Culture: 10–14 days	☐ Culture:3–4 weeks	☐ Culture:24 -48 hours
Fetal loss	0.5–1%	0.5%	1–2%
Accuracy	Accurate; may need amniocentesis for confirmation	Highly accurate	Highly accurate
Termination of pregnancy when indicated	1st trimester—safe	2nd trimester—risky	2nd trimester–risky
Maternal eff ects following termination of pregnancy	Very little	More traumatic; physically and psychologically	Same as amniocentesis

NONINVASIVE Method of Prenatal Testing from Maternal Plasma/Blood

Fetal DNA comes in the maternal circulation from the placenta. Fetal DNA can be detected in maternal plasma and whole blood from the first trimester onward. This is rapidly cleared from the maternal circulation after delivery. Cell-free fetal DNA (cff-DNA) is a reliable source for prenatal diagnosis.

Approximately 5% of cell free DNA in the maternal blood is fetal. The cff DNA is a reliable source for prenatal diagnosis. The amount of cff-DNA in maternal blood increases with gestational age. The test is generally done from 10 weeks of pregnancy.

Testing for cff-DNA is highly sensitive and specific. Detection rates for fetal trisomy 13, trisomy 18 and trisomy 21 are greater than 98%, with a very low false-positive rate (< 0.5 %). However, a woman with a positive test result should be referred for genetic counseling and should be offered invasive prenatal diagnosis for confirmation of test results. (ACOG-2012).

Conditions for Diagnosis with cff-DNA

1. Fetal Rh-D typing using cff-DNA to determine fetal blood group status. This is done without amniocentesis .
2. Single gene disorders can be diagnosed when the father has a mutation and that is not present in the mother (Marfan syndrome, cystic fibrosis).
3. Fetal aneuploidy: Trisomy 21.

Intact fetal cells: Fetal trophoblasts, lymphocytes, granulocytes or nucleated red blood cells can be isolated from maternal blood. Analysis of intact fetal cells by FISH with specific chromosome probes can diagnose fetal aneuploidy for other chromosomes besides trisomy 21. However, intact fetal cells are rare in maternal blood (1 per 1–10 million maternal cells).

BIOPHYSICAL:

Ultrasonographic examination of the fetus in the early (10–14 weeks) pregnancy can detect fetal anomalies. Crown-rump length (CRL) smaller than the gestational age is associated with the risk of chromosomal anomalies (trisomy or triploidy). Increased nuchal translucency (NT) at 10–14 weeks is associated with many chromosomal abnormalities (trisomy, monosomy, triploidy).

Detection rate is about 70–80% with a false-positive rate of 5–6%. Absence of nasal bone (NB) on USG at 10–12 weeks is associated with fetal Down's syndrome. When NB and NT were combined, detection rate of trisomy 21 was 92% with a false positive rate of 3.5%.

U/S Features in Down Syndrome

Thickened nuchal fold

Choroid plexus cyst

Short femur length

Short humeral length

Echogenic bowel

Echogenic intracardiac focus

Renal pyelectasis

Prenatal diagnosis: Biochemical and Biophysical screening tests

	β-hCG+ PAPP-A + NT	MSAFP	MSAFP, hCG, uE3(triple test)	MSAFP, uE3,hCG, inhibin A	soft tissue Marker (nuchal translucency; nasal Bone)
Time (weeks)	11–14	15–20	15–18	15–20	11–14
Observation	β-hCG (↑), PAPP-A (↓)	MSAFP (↑)	MSAFP (↓); uE3 ↓; hCG (↑)	MSAFP (↓); uE3 ↓; hCG (↑); Inhibin(↑)	Nuchal thickness (NT) > 3 mm, Nasal bone absent
Anomaly to detect	Down's syndrome	Open neural tube defects	Down's syndrome	Down's syndrome	Down's syndrome turner's syndrome and others
Comment	A cut off value 1 in 300 is screen positive	Cut-o level off 2.5 MOM can detect 90% of anencephaly, 80% open spina bifda	A cut-off value of 1 in 200 is screen positive	Detection rate is high	Detection rate of Down's syndrome is high (92%), when NT and NB are combined
Detection rate	85–92%	85%	73%	85–92%	85–92%
False-positive rate	5%	3–5%	5%	0.9%	3–5%

Magnetic resonance imaging (MRI): Information superior to ultrasonography could be obtained

Peri-implantation genetic diagnosis (PGD) is done by: (a) polar body biopsy, (b) blastomere biopsy (from 6–8 cell embryo) and (c) trophectoderm biopsy (5–6 days blastocyst). Diagnostic accuracy in PGD is high (98–99%) both for cytogenetic and single gene disorders. FISH technique is used for detection of aneuploidy, translocation and other chromosomal rearrangements. PGD may be preferred to usual prenatal diagnosis (CVS or amniocentesis) where pregnancy termination is not accepted.

Polar body biopsy: It is done by removing the first or second polar body in the preconceptional phase.

Paternal genotype is not assessed here.

Blastomere biopsy: One or two cells are aspirated through a hole made in the zona pellucida by mechanical, laser or chemical means. This does not affect the normal embryonic development.

Fetal therapy: Intrauterine fetal tranfusion for fetal anemia (alloimmunization, thalassemia) is done.
Fetal medical therapy is done for various conditions through maternal medication. Medicines are carried transplacentally to the fetus. Maternal oral therapy with propylthiouracil for fetal hyperthyroidism, digoxin or flecainide for fetal tachyarrhythmias and oral dexamethasone for congenital adrenal hyperplasia of a female fetus have been found effective. Fetal stem cell transplantation and fetal gene therapy could be used for many hematological, metabolic, immunological and inherited diseases. Intrauterine fetal surgery has been attempted in few selected cases. Common fetoscopic surgeries done are: laser therapy for TTTS ,cystoscopic laser for posterior urethral valves, fetal tracheal occlusion for congenital diaphragmatic hernia and release of amniotic bands.