Obstetrics – Dr. Hana – Lecture 2 – Prenatal Diagnosis

Introduction
Congenital abnormalities account for 20-25% of perinatal deaths. Now, many genetic and other disorders can be diagnosed early in pregnancy. Prenatal diagnosis uses various noninvasive and invasive techniques.

Prerequisites of Prenatal Diagnosis
Prenatal diagnosis is recommended in the following cases:
1. The pregnant woman is 35 years or older at the time of delivery.
2. She or her parents have had a previous child with a chromosomal abnormality.
3. She has a history of recurrent abortions, or her husband’s previous wife experienced several miscarriages.
4. A history of parental consanguinity is present.
5. The couple is known to be carriers of a chromosomal translocation.
6. The pregnant woman is affected with type 1 diabetes mellitus, epilepsy, or myotonic dystrophy.
7. She is exposed to viral infections, such as rubella or cytomegalovirus.
8. The mother is exposed to excessive medication or to environmental hazards.
9. In her or her spouse's family, a history of Down syndrome or some other chromosomal abnormality is present.
10. A history of single gene disorder is present in her or her spouse's family.
11. The fetus is detected to be at increased risk for a NTD.

Benefits of Prenatal Diagnosis
The benefits of prenatal diagnosis are as follows:
1. Prenatal diagnosis determines the outcome of pregnancy.
2. It is helpful for couples to decide whether to continue the pregnancy.
3. It indicates possible complications that can arise at birth process.
4. Prenatal diagnosis is helpful for the management of remaining weeks of pregnancy.
5. It prepares the couple for the birth of a child with an abnormality.
6. Prenatal diagnosis can be helpful for the improvement of the outcome of pregnancy using fetal treatment.

Noninvasive techniques
1. Fetal visualization
   • Ultrasound
   • Fetal echocardiography
   • MRI
   • Radiography
2. Screening for neural tube defects (NTDs) - Measuring maternal serum alpha-fetoprotein (MSAFP)
3. Screening for fetal Down syndrome
   • Measuring MSAFP
   • Measuring maternal unconjugated estriol
   • Measuring maternal serum beta-human chorionic gonadotropin (HCG)
4. Separation of fetal cells from the mother’s blood
Invasive techniques

1. Fetal visualization
   - Embryoscopy
   - Fetoscopy

2. Fetal tissue sampling
   - Amniocentesis
   - Chorionic villus sampling (CVS)
   - Percutaneous umbilical blood sampling (PUBS)
   - Percutaneous skin biopsy

3. Other organ biopsies, including muscle and liver biopsy

4. Preimplantation biopsy of blastocysts obtained by in vitro fertilization

Cytogenetic investigations

Detection of chromosomal aberrations
Fluorescent in situ hybridization

ULTRASONOGRAPHY

First Trimester Findings

1. Normal evaluation of fetal number
2. Location of the placenta
3. Evaluation of the fallopian tubes and ovaries
4. Location of the pregnancy (intrauterine or ectopic)
5. Measurement of the crown-rump length (CRL) or the gestational sac diameters
6. Nuchal translucency

Second and Third Trimester Findings

1. Head and intracranial anatomy
2. Face
3. Heart and chest
4. Abdomen
5. Kidneys
6. Spine
7. Extremities
8. Placenta, amniotic fluid, uterus and
9. Other structures

Ultrasound findings associated with chromosome abnormalities, e.g. Down syndrome:
- Nuchal edema or translucency
- Hyperechoic bowel
- Pyelectasis
- Choroid plexus cysts
- Short humerus and femur lengths
Fetal echocardiography
Fetal echocardiography can be performed at 15 weeks' gestation and beyond. When this technique is used with duplex or color flow Doppler, it can identify a number of major structural cardiac defects and rhythm disturbances.

Fetal echocardiography is recommended in:
1. Maternal diseases, such as diabetes or phenylketonuria associated with fetal structural heart defects, in particular heart blocks, such as lupus or other immune disorders
2. Alcohol or drug consumption by mother during pregnancy
3. Maternal rubella infection during pregnancy

MRI
MRI is a fetal imaging technique that uses powerful magnets and radio waves to construct images of the body, but, because of fetal movements, its application has been limited.

Radiography
The fetal skeleton can be visualized by radiography from 10 weeks' gestation onward. This technique is used for the diagnosis of inherited skeletal dysplasias, particularly osteochondrodysplasia, in the second and third trimesters. Because of the dangers of radiography to the fetus, this technique rarely is used.

Measuring maternal serum alpha-fetoprotein
The developing fetus has 2 major blood proteins, albumin and alpha-fetoprotein (AFP), while adults have only albumin in their blood. The MSAFP level can be used to determine the AFP levels from the fetus. AFP is produced by the yolk sac and later by the liver; it enters the amniotic fluid and then the maternal serum via fetal urine.

Screening for fetal Down syndrome (triple screening)
- Measuring MSAFP (decreased)
- Measuring maternal unconjugated estriol (decreased)
- Measuring maternal serum beta-human chorionic gonadotropin (HCG)
- Others
  - Pregnancy associated plasma protein - A (PAPP-A) (decreased)
  - Inhibin A (increased)

Maternal serum alpha-fetoprotein
- Glycoprotein
- Yolk sac (early), liver and GI tract (late)
- Unknown function
- 14-22 wk, highest sense 16-18wk
• 2.0 or 2.5 MoM is upper limit of normal

**Human chorionic gonadotropin**
• Glycoprotein
• Syncytiotrophoblast
• High level in Trisomy 21

**Unconjugated estriol (UE3)**

DHEAS
16 alpha-hydroxy-DHEAS

Estriol
• level indicated well-being of the fetus
• low level in Trisomy 21

**Triple screening**

*Accuracy depends on*
– pregnancy dating
– patient information
– screening parameters utilized by laboratory

• **definitive diagnosis can be made only by**
  chromosome analysis of fetal tissues obtained by CVS, amniocentesis or PUBS

**Screening for neural tube defects**

*It is recommended if the following are present:*

1. Ultrasound findings indicate NTDs.
2. A child with NTDs is already in the family.
3. A family history of NTDs exists, especially a mother with NTDs.
4. The mother has type 1 diabetes mellitus during pregnancy.
5. Maternal exposure to drugs, such as valproic acid, is associated with NTDs.
6. Elevated level of MSAFP is present.

In the condition of an open NTD (eg, anencephaly, spina bifida) and abdominal wall defects in the fetus, AFP diffuses rapidly from exposed fetal tissues into amniotic fluid, and the MSAFP level rises. However, the MSAFP levels also increase with gestational age, gestational diabetes, twins, pregnancies complicated by bleeding, and in association with intrauterine growth retardation.

The MSAFP test has the greatest sensitivity between 16-18 weeks' gestation, but it also can be performed between 15-22 weeks' gestation. A combination of the MSAFP test and ultrasonography detects almost all cases of anencephaly and most cases of spina bifida.

Also, a NTD can be distinguished from other fetal defects, such as abdominal wall defects, by the use of an acetylcholinesterase test carried out on amniotic fluid obtained by amniocentesis.
If the level of acetylcholinesterase rises along with AFAFP, it is suspected as a condition of a NTD.

**Separation of fetal cells from the mother's blood**

Fetal blood cells make access to maternal circulation through the placental villi. These cells can be collected safely from approximately 18 weeks' gestation onward, although by successful procedures, these cells can be collected at 12 weeks' gestation. The fetal cells can be sorted out and analyzed by different techniques.

Fetal blood cells can be analyzed for the diagnosis of genetic disorders using molecular genetic techniques by isolating DNA and amplifying it by polymerase chain reaction (PCR). Fetal cells separated from a mother's blood have been successfully used in the diagnosis of cystic fibrosis, sickle cell anemia, and thalassemia in a fetus.

**Invasive Techniques - Fetal visualization**

**Embryoscopy**

Embryoscopy is performed in the first trimester of pregnancy (up to 12 weeks' gestation).

In this technique, a rigid endoscope is inserted via the cervix in the space between the amnion and the chorion, under sterile conditions and ultrasound guidance, to visualize the embryo for the diagnosis of structural malformations.

**Fetoscopy**

Fetoscopy is performed during the second trimester (after 16 weeks' gestation).

In this technique, a fine-caliber endoscope is inserted into the amniotic cavity through a small maternal abdominal incision, under sterile conditions and ultrasound guidance, for the visualization of the embryo to detect the presence of subtle structural abnormalities.

It also is used for fetal blood and tissue sampling. Fetoscopy is associated with a 3-5% risk of miscarriage; therefore, it is superseded by detailed ultrasound scanning.

**Fetal tissue sampling - Amniocentesis**

Amniocentesis is an invasive, well-established, safe, reliable, and accurate procedure performed between 14-20 weeks of pregnancy. Amniocentesis is advised for pregnant women at 35 years or older for detection of chromosomal abnormalities in the fetus.

It is performed under ultrasound guidance. A 22-gauge needle is passed through the mother's lower abdomen into the amniotic cavity inside the uterus, and 10-20 mL of amniotic fluid that contains cells from amnion, fetal skin, fetal lungs, and urinary tract epithelium are collected.

These cells are grown in culture for chromosomal, biochemical, and molecular biologic analyses. Supernatant amniotic fluid is used for the measurement of substances, such as AFAFP, hormones, and enzymes. The results of cytogenetic and biochemical studies on amniotic cell cultures are more than 90% accurate.

In the third trimester of pregnancy, the amniotic fluid can be analyzed for determination of fetal lung maturity. Risks with amniocentesis are rare but include 0.5-1.0% fetal loss and maternal Rh sensitization.
Fetal tissue sampling - Chorionic villus sampling

CVS is performed very early in gestation between 9-12 weeks, ideally at 10 weeks' gestation. A catheter is passed through the cervix or through the abdominal wall into the uterus under ultrasound guidance, and a sample of chorionic villi surrounding the sac is obtained. The villi are dissected from the decidual tissue, and chromosome analysis is carried out on these cells to determine the karyotype of the fetus.

The major advantage of CVS over amniocentesis is getting quick results and its use in early pregnancy. Abnormalities can be identified at an early stage, and more acceptable decisions about termination of the pregnancy can be taken. Abortion is also much safer at this early stage.

A disadvantage of CVS as compared to amniocentesis is a 2-3% risk of causing miscarriage, and, rarely, CVS can result with limb defects in the fetus. Maternal sensitization is possible. A higher rate of maternal cell contamination and confined placental mosaicism with CVS may result in diagnostic ambiguity, leading to the need for additional invasive diagnostic tests.

Percutaneous umbilical blood sampling

PUBS is also known as cordocentesis. It is a method for fetal blood sampling and is performed after 16 weeks' gestation. A needle is inserted into the umbilical cord under ultrasound guidance, and fetal blood is collected from the umbilical vein for chromosome analysis and genetic diagnosis.

An advantage of PUBS is the rapid rate at which lymphocytes grow, allowing prompt genetic diagnosis. This technique is also useful for evaluating fetal metabolism and hematologic abnormalities.

Percutaneous skin biopsy

To prenatally diagnose a number of serious skin disorders, fetal skin biopsies are taken under ultrasonic guidance between 17-20 weeks' gestation.

Other organ biopsies, including liver and muscle biopsy

Fetal liver biopsy is needed to diagnose an inborn error of metabolism, between 17-20 weeks' gestation under ultrasound guidance.

Fetal muscle biopsy is carried out under ultrasound guidance at about 18 weeks' gestation to analyze the muscle fibers histochemically for prenatal diagnosis of Becker-Duchenne muscular dystrophy.

Preimplantation biopsy of blastocysts obtained by in vitro fertilization

Techniques are being developed to test cells obtained from biopsy of early cleavage stages or blastocysts of pregnancies conceived through in vitro fertilization. These techniques will be helpful for selective transfer and implantation of those pregnancies into the uterus that are not affected by a specific genetic disorder. This approach will be more acceptable to those couples who oppose abortions.
Cytogenetic Investigations - Detection of chromosomal aberrations

Chromosomal aberrations, such as deletions, duplications, translocations, and inversions diagnosed in affected parents or siblings, can be detected prenatally in a fetus by chromosomal analysis. This analysis can be undertaken on fetal cells obtained through such techniques as amniocentesis and CVS.

Fluorescent in situ hybridization

FISH uses different fluorescent-labeled probes, which are single-stranded DNA conjugated with fluorescent dyes and are specific to regions of individual chromosomes. These probes hybridize with complementary target DNA sequences in the genome and can detect chromosomal abnormalities, such as trisomies, monosomies, and duplications.