Review Article

PRENATAL DIAGNOSIS OF GENETIC DISORDERS

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Prenatal diagnosis of genetic disorders has been possible because of great advances in techniques of obtaining fetal tissues as well as development in cytogenetics, biochemical techniques and recombinant DNA technology. The techniques currently in use or under investigation for prenatal diagnosis are outlined in *Tables I* and *II.*

Amniocentesis

It is the withdrawal of amniotic fluid from the amniotic sac surrounding the fetus(l). If amniocentesis is done at 15-16 weeks of gestation the process is called conventional(2), and if between 11-15 weeks early amniocentesis; the latter procedure is still experimental. Early amniocentesis is considered to be useful for cytogenetic studies(3).

Chorionic Villus Sampling (CVS)

This procedure is one of the earliest methods of obtaining fetal tissues for karyotype, DISFA studies and enzyme assay(4). The procedure is done at 8-11 weeks of gestation. The transcervical sampling involves insertion of various types of catheters transcervically under ultrasound guidance for aspiration of chorionic villi. Transabdominal CVS is preferred as it can be done even in the second trimester of pregnancy and is associated with lesser risk of infection, bleeding and pregnancy loss(5). CVS can be done in the second or third trimester of pregnancy by direct aspiration of placental villi through transabdominal route under ultrasound guidance.

Coelocentesis is a new technique that avoids puncture of the amnion. It involves puncture of the chorion and aspiration of the coelomic fluid from the extra-embryonic coelom. This procedure has not been used in clinical practice(6).

Fetal Blood Sampling

Initially this procedure was done by passing a fetoscope transabdominally at 18-20 weeks, under ultrasound guidance. It never gained popularity because of its difficulty and high rate of fetal loss (approximately 4%). Fetal blood sampling has been replaced by cordocentesis *i.e.,* withdrawing blood from the umblical vein under ultrasound guidance(7). Cordocentesis has not only helped in the prenatal diagnosis of genetic disorders, but also in understanding of fetal physiology, development and metabolism. The most important indications for cordocentesis include: *(i)* rapid karyotyping for chromo-

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TABLE I-*Techniques for Prenatal Diagnosis*

Fetal Tissues	Technique	Timing (weeks)	Studies Done on Tissues	Risk
Amniotic fluid	*(a)* Conventional amniocentesis	15-16	AFP *I* ACHE hCG	Abortion, needle puncture injuries, placental abruption, chorio amnionitis, abruption, preterms labour.
	(b) Early amniocentesis	11-14		
Amniocytes	*(a)* Conventional amniocentesis	15-16	Cell culture for karytopes, enzyme assay, DNA studies, FISH	
	(b) Early amniocentesis	11-14		
Chorionic Villi	*(a)* Transvaginal	8-11	Biochemical, chromosmal, DNA	2% fetal loss, limb defects, mosaicism and maternal bleedding
	(b) Transabdominal	12-24		
Fetal blood	*(a)* Fetoscopic aspiration	18-20	Coagulation factor Immunoglobulin antibodies estimation; DNA and enzyme study; karyotype, FISH.	1 % fetal loss, Rhesus sensitization, fetal infection, PROM
	(b) Cordocentesis	16-20		
Fetal liver	*(a)* Fetoscopic biopsy		Enzyme assay as in OTC deficiency	
	(b) Percutaneous biopsy	18-20		?
Fetal skin	*(a)* Fetoscopic biopsy	18-20	Histopathology	
	(b) Percutaneous biopsy			?
Fetal muscle	*(a)* Fetoscopic biopsy	18-20	Histopathology	
	(b) Percutaneous biopsy			?
Maternal serum	Maternal blood	12-14	AFP/UE3/hCG	Nil
Fetal Cells in maternal circulation	Flow cytometry, *PCRI* monoclonal antibodies	1st trimester	FISH, fetal sexing DNA tests	Nil
Pre-implanation embryo biopsy	IVF Biopsy of blastocysts	4-8 cell stage blastocyst	DNA, PCR, enzyme assay	?

AChE-Acetylcholine esterase; OTC-Ornithine transcarbamylase; PROM-Premature rupture of membranes.

T ABLE II-*Fetal Visualization Techniques*

Techniques	Route	Timing	Risk to fetus
Embry-oscopy	Trans-vaginal	First tri-mester	?
Fetos-copy	Trans-abdominal	18-20 weeks	3%
Embryo-fetoscopy	Trans-abdominal	First tri-mester	?
Ultra-sound	Trans-abdominal	15-20 weeks	nil
	T rans-vaginal	First trim-ester	nil
MRI		All trim-esters	?
Radio-graphy		Late second and third trimester	

somal disorders and where either amniotic fluid or CVS have shown chromosomal mosaicism; *(it)* diagnosis and treatment of Rh isoimmunization; *(iii)* immunoglobulin deficiency; *(iv)* clotting factor deficiencies; *(v)* hemoglobinopathies; *(vi)* fetal platelet abnormalities; *(vii)* evaluation of nonimmune hydrops fetalis(8).

Fetal Skin, Liver and Muscle Sampling

Fetal skin sampling was initially performed by fetoscopy, which has been replaced by percutaneous insertion of biopsy forceps under ultrasound guidance. Conditions which can be detected by fetal skin sampling include anhidrotic and hypohidrotic ectodermal dysplasia, epidermolysis bullosa, oculocutaneous albinism, and Sjogren-Larsson syndrome(9).

Fetal liver biopsy may rarely be necessary to diagnose inborn errors of metabolism limited to hepatic parenchymal enzyme abnormalities. Attempts have also been made to diagnose muscular dystrophy by prenatal muscle biopsy(10). Recent advances in DNA technology have however allowed reliable diagnosis of muscle dystrophy and reduced the need for fetal muscle biopsies.

Techniques for Fetal Imaging

Ultrasound : The role of ultrasound in prenatal medicine has expanded over the past two decades. As a result the possibilities for diagnosis and *in utero* therapy and surgical interventions is now possible. The timing for ultrasound examination for various structural anomalies is shown in *Table III* (11).

Prenatal diagnosis of central nervous system malformations especially that of

TABLE III-*Optimal Timing for Ultrasound Examination*

Indication	Timing
Anencephaly	10-12 weeks
Spina bifida	16-18 weeks
Hydrocephaly/ microcephaly	16 weeks onward
Cardiac abnormalities	18-22 weeks
Limb abnormalities	10 weeks onward
Renal disease	16 weeks onward
Face and mouth-anomlies	18-22 weeks
Anterior abdominal-wall	16-18 weeks

neural tube defects is easy on ultrasound imaging (*Table IV).* Many skeletal dysplasias and chromosomal anomalies including Down syndrome can be diagnosed on ultrasonography *(Table V).* The finding of a strawberry shaped head, choroid plexus cysts, facial cleft, micrognathia, exomphalocele and complex hand and feet malformations suggest trisomy 18(12).

The indication for fetal echocardiography include family history of congenital heart diseases, diabetic mother, intrauterine infection, teratogenic exposure, nonimmune hydrops and unexplained polyhydramnios(13). Transvaginal and transabdominal ultrasonography done at 11-14 weeks and 18-20 weeks respectively help to detect congenital heart disease(14).

TABLE IV-*Prenatal Diagnosis in Neural Tube Defect*

MSAFP	Increased (2nd trimester)
Ultrasound	Transabdominal (2nd trimester) Transvaginal ultrasound (1st trimester)
Anencephaly	Absence of vault
Spina bifida	Spine anatomy-splaying of spine Lemon sign" Banana sign.... Swelling, hydrocephalus, lower limb defects
Amniotic fluid	Raised AFP, acetylcholinesterase
Chromosomal studies	To exclude NTD, as a part of chromosomal disorder.

* Lemon sign: Forward scalloping of - the frontal aspect of the fetal head

** Banana sign: Banana shaped abnormal cerebellum.

Esophageal atresia, pulmonary cystic adenomatoid malformations, pulmonary hypoplasia, diaphragmatic hernia, hyper-echogenic bowel, obstructive lesiosn, ventral wall defects can be detected during second and third trimester of pregnancy.

Fetoscopy: A fine caliber endoscope is inserted into the uterus. The direct visualization of the fetus is possible and fetal blood sampling can be done. Because of the high risk of spontaneous abortion associated with fetoscopy, this technique is rarely used.

Etnbryoscopy: This is an experimental technique used in the first trimester of pregnancy. A rigid endoscope inserted through the cervix into the space between the amnion and chorion is used to visualize the embryo and for diagnosis of structural malformations(15).

Embryofetoscopy: Transabdominal embryofetoscopy is a new technique being evaluated for its utility in the first trimester diagnosis of structural abnormalities of the fetus. This technique has been possible because of availability of new submillimetric fiberoptic endoscope(16).

Magnetic Resonance Imaging: The clinical applications of this technique are limited due to fetal movements and the cost involved in this mode of investigations(17).

Radiography: The indications for con-

TABLE V-*Prenatal Diagnosis in Down Syndrome*

Maternal serum markers		
AFP	- decreased (14-16 weeks)	
UE3	- decreased (14 weeks)	Triple test:
hCG	- increased (14 weeks)	detection rate is 60%
PAPP-A	- increased 1st trimester	
Neutrophil alkaline phsophatase-increased		
Amniocentesis		
Early	- Karyotype on cultured amniocytes	
Conventional	- FISH on uncultured cells	
Cordocentesis	(18 weeks)	Fetal lymphocytes; Culture for karyotype; FISH on non-dividing fetal cells
CVS	(8-11 weeks)	Direct preparation for karyotype, short term culture
Ultrasound	(11-20 weeks)	Nuchal fold > 4 mm, double .bubble in abdomen, short femur, renal pyelectasis, clinodactyly, macroglossia
Fetal echocardiography		Atrio-ventricular canal defects
Fetal cells in maternal circulation		FISH

Figures in parenthesis indicate appropriate time for that test.

ventional radiographs are limited to diagnosis of bone dysplasias in second or third trimester(17)..

Prenatal Cytogenetics

All chromosomal aneuploidies and the majority of structural chromosomal abnormalities (deletion, translocation) can be detected prenataly. Cells used for prenatal cytogenetic analysis includes amniocytes obtained by second trimister amniocentesis, chorionic villi obtained by transcervical or transabdominal CVS or fetal blood sampling. Indications for prenatal cytogenetic analysis include advanced maternal age, previous child with aneuploidy, parental chromosome rearrangements and fetal abnormalities detected by ultrasound. The result of fetal karyotype are available within 72 hr with cord blood samples and CVS, and 2-3 weeks with amniotic fluid.

The combination of molecular genetics and cytogenetics techniques (molecular cytogenetics) has given rise to the powerful new technology of fluorescent *in situ* hybridisation (FISH)(18). Briefly, a fluoroscently labelled DNA probe is hybridised to a standard chromosome preparation on a microscopic slide. This method is useful in detecting chromosomal abnormalities, both numerical and microdeletion syndromes. For FISH

technique chromosome specific probes (alpha satellite probe) which hybridise with pericentromeric region of the chromosome are used. This technique is used for detecting chromosomal aneuploidy, that often involve X, Y, 13,18 and 21 chromosomes.

A set of probes derived from whole single chromosome containing repetitive sequences that span the length of a chromosome can be prepared and labelled with fluorochromes (chromosome painting). A further use of FISH technique is the generation of region specific chromosome probes for the diagnosis of contiguous gene syndromes(19). Contiguous gene syndromes involves the loss of genes that may be functionally unrelated but are contiguous along the chromosome. These syndromes include Prader Willi syndrome, Miller-Dicker syndrome, Angelman syndrome and Di George syndrome. FISH has also been used for the detection of Duchenne or Becker muscular dystrophy carrier status in females whose affected male relatives have a known specific gene deletion.

DNA Based Prenatal Diagnosis

This clinical application is based upon the fact that DNA complement is generally identical in every cell of the body, and therefore a hereditary defect detectable at the DNA level, should be found in all nucleated cells from that individual(20,21). Any nucleated fetal cells can be used to obtain DNA for diagnosis.

A. Techniques for direct mutation analysis include:

(a) Analysis of' the disorder through restriction enzyme: Restriction endonucleases are bacterial enzymes that recognise and cleave short specific base sequences in double stranded DNA and are used to identify mutant genes, deletions within gene,, or characterize DNA polymorphism.

(b) Polymerase Chain Reaction (PCR): When the molecular basis of a disease is known, the precise test can be designed. Most rely on the ability to amplify quickly and efficiently a specific region of DNA, using the PCR. This technique allows simple and rapid synthesis of microgram quantities of DNA copies from template segments present in amounts as small as a single molecule. Various in herited diseases including alpha 1 antitrypsin deficiency, Duchenne mus cular dystrophy, phenylketonuria and fragile X syndrome can be diagnosed by PCR.

(c) Allele Specific Oligonucleotide Probes: This is an alternative way to detect a known point mutation. The technique uses two synthetic oligonucleotides, one specific for a normal gene and the other specific for the known genetic mutation. In autosomal recessive disorders, in the affected individual there will be two copies of mutated genes, while the normal individual will have two normal genes and the carrier one mutated and one normal gene e.g., sickle cell disease, alpha 1 antitrypsin deficiency.

(d) Expanding Trinucleotide Repeats: This form of mutation is seen in 3 genetic disorders, fragile X syndrome, myotonic dystrophy and Huntigton's disease. In these disorders normally polymorphic trinucleotide repeat sequences found at the disease locus ex-

pand in affected individuals beyond the normal size range. In fragile X syndrome, CGG repeats extend to hundred or thousands of the triplets. Detection of expanding trinucleotide repeats is relatively simple. The simplest method is to amplify across the expanding region(22,23). When the number of repeats is very large the expansion can be detected by DNA hybridization with a probe that is located close to the region of expansion.

Other techniques for evaluation of mutations include dot blot analysis and amplification refractory mutation systems.

B. Techniques for indirect mutation analysis include:

(a) Linkage: In most genetic disorders the mutation is unknown. By using certain markers, that are close to mutant; gene, one can indirectly infer about the presence of mutant gene because of its proximity to the probe.

(b) Restriction Fragment Length Polymorphism (RFLP): This is a type of molecular marker produced when specific enzymes are used to cut the DNA. Measurements of the fragment length to which a DNA probe will hybridize can be used to track the transmission of a variation in the DNA (polymorphism) through a family. Linkage of an RFLP to the gene of interest can be used for genetic diagnosis.

About 80-85% of the thalassemic mutations can be detected by this method. This can be used as indirect marker to detect single base substitution or deletion in the beta globin gene. One major disadvantage of these method is that linkage analysis requires a study of multiple family members from several generations.

The other types of polymorphisms becoming increasingly useful in diagnostic linkage analysis are microsatellites or simple sequence repeats(24).

Biochemical Screening

Alpha-Feto Protein (AFP)

AFP is a glycoprotein containing about 4% carbohydrate which can be detected in the human fetus by 29 days of life. It is produced initially both in the yolk sac and the liver, but the hepatic contribution increases steadily. Its exact function is not known, but it acts as a carrier protein and has an immunological role. AFP is excreted into fetal serum and the main source of AFP in the amniotic fluid is the fetal urine; fetal skin may also allow passage of a small amount of AFP. By 10 weeks of gestation AFP increases, peaks at 13 weeks and decreases thereafter by 12% every week. AFP is increased in neural tube defects and decreased in chromosomal aneuploidy like Down syndrome.

During pregnancy the maternal serum level of AFP (MSAFP) increases. AFP is transferred to the maternal circulation by two routes, across the placenta directly from fetal serum and though the fetal membranes from the amniotic fluid. The median MSAFP levels measures about 25 ng/ml at 16 weeks and increases by about 15% per week until about 30 weeks, plateaus for the next 5 to 6 weeks and then falls rapidly.

Increased levels of MSAFP are seen in pregnancies with multiple gestation, neural tube defects, placental abnormalities, maternal disease like hepatocellu-

lar carcinoma, viral hepatitis and systemic lupus erythematosus. The levels of MSAFP are decreased when the fetus has Down syndrome or other trisomies(25).

Unconjugated Estriol (UE 3)

UE 3 produced by the fetal adrenal gland is converted by the placenta and conjugated by the maternal liver. UE 3 levels in maternal serum are approximately 25% lower in pregnancies with Down syndrome. Measurement of UE3 levels in the maternal serum distinguish between Down syndrome and unaffected pregnancy as early as 9 to 11 weeks of gestation.

Other proteins which can be detected in the maternal serum in Down syndrome include human chorionic gonadotropin(25), pregnancy associated placental protein A(26) and neutrophil alkaline phosphatase *(Table V).*

Fetal Cells in Maternal Blood

The nucleated fetal cells circulating in the maternal blood can be utilized for DNA extraction arid prenatal tests(27). Such tests can be done in the first trimester for population screening. They carry no risk of maternal infection or fetal miscarriage. The fetal cells isolated include nucleated red blood cells, leukocytes and trophoblasts. These cells can be isolated from maternal blood by flow cytometry, monoclonal antibodies or PCR.

Prenatal Diagnosis Before Implantation

The diagnosis of genetic disorders in preimplantation embryo is being evolved as a new specialty. Such a diagnosis would allow the selection and transfer of only healthy zygotes to the uterus.

Access to preimplantation embryo is possible by either non-surgical uterine lavage or *in vitro* fertilization(28). The blastocyst with 100-200 cells obtained by the uterine lavage is subjected to microbiopsy. A small slit is cut into the zona pellucida of blastocyst with a micromanipulator. As the blastomere cells herniate through the incision they are excised from the embryo surface and subjected for genetic diagnosis. After the microbiopsy, the remaining blastomere may be cryopreserved in liquid nitrogen. If the result of microanalytic diagnostic technique on biopsy specimen proves to be normal, then the preimplantation embryo is replaced into the uterine cavity.

The cells obtained from the biopsy specimen may be cultured to increase the yield. The biopsied specimen of preimplantation embryo is subjected to various techniques for genetic diagnosis. Rapid advances in recombinant DNA, monoclonal antibodies, flow cytometry and cytogenetic techniques have made it possible to subject even a single cell for genetic diagnosis. Micromanipulation of the embryo, though no adverse effects are reported, is potentially a teratogenic procedure.

DNA Based Diagnosis Available in India

Prenatal diagnosis using DNA technology are being offered for betathalassemia and Duchenne muscular dystrophy at the Genetic Unit, Department of Pediatrics. All India Institute of Medical Science, New Delhi 110 029.

The cost for the DNA based study is approximately Rs. 1500/- (Dr. I.C. Verma, New Delhi, personal communication). The Birth Defect Centre, A-ll, Elco Market Road, Bandra (W), Bombay 400 050 also provides similar services, though at comparatively higher rates.

Diagnosis of beta Thalassemia

Prenatal diagnosis is done by DNA studies on the CVS obtained at 9-12 weeks, amniotic fluid cells obtained at 14-15 weeks, or fetal blood obtained at 18 weeks. The desired method is CVS. Initially it has to be established that both parents are carriers. The birth of an affected child makes them obligate carriers. The blood level of Hb A2 are raised in carriers. Blood is collected in a sterile EDTA tube from both parents and the affected child. The affected child should not have received any blood transfusion in the past 3-4 weeks. All the blood samples should preferably be collected before the CVS. A date is fixed with an obstetrician for CVS. Laboratory diagnosis using DNA technology takes about 10-14 days.

Diagnosis of Duchenne Muscular Dystrophy

Definitive diagnosis in the affected case is the first step in the diagnosis. Next decide whether mother is a carrier or not. This is done by pedigree analysis and high values of creatinine phosphokinase. However, it must be remembered that during pregnancy creatinine phosphokinase values are low. At least two enzyme determinations must be made one week apart.

The DNA of the affected child must be examined to determine the exact defeet in the gene. It is ideally done before mother is pregnant. This will tell whether prenatal diagnosis is possible or not. During pregnancy at about 10-11 weeks a CVS is obtained and the sex of the fetus determined. If the fetus is female, no further tests are done. If fetus is male, the DNA extracted from CVS is tested for abnormality of the gene.

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