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INVITED SPEAKERS

Prepregnancy Assessment and Intervention

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Prepregnancy is a physiological condition. But for some mothers and their offsprings it carries potential harm. Identifying such pregnancies permits preventive measures. Good diet, exercise, avoidance of drugs, tobacco and alcohol excess are necessary for fetal health. Diabetes, hyperthyroidism and cardiac diseases should be adequately treated before planning a pregnancy. Certain antiepileptic medications and anticoagulants have to be avoided during pregnancy. Some women by virtue of advanced age, ethnicity, pedigree, family history, radiation exposure, personal history etc. are likely to have malformed fetuses. Such women should be identified, studied genetically and possible preventive measures taken to avoid births of malformed children. In fact, most of such births are avoidable. Simple measures like folic acid supplementation, rubella immunization and life style changes can avoid births of congenitally malformed children. Proper assessment before pregnancy permits a couple to have reproductive choices planning a pregnancy, postponing a pregnancy, postponing a pregnancy till certain preventive measures are taken and avoiding a pregnancy altogether.

Prenatal Diagnosis permits termination of pregnancy and fetal therapy. Gamete donation, in-vitro fertilization and preimplantation genetic diagnosis are never options now available to couples otherwise destined to produce babies with congenital malformations. Prepregnancy assessment, good counseling, and proper preventive intervention would eliminate births of malformed children.

Birth Defects, CNS and IQ: Role of Folic Acid and Hypothyroxinemia

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First Trimester Ultrasound for Congenital Malformations

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First trimester anomaly scan can be performed abdominally or vaginally. Transvaginal scan has a higher sensitivity than abdominal and has revolutionized the assessment of first trimester fetus (1). First trimester scanning has a number of advantages: to establish fetal viability, confirm multiple pregnancies and determine chorionicity, accurate gestational dating, nuchal translucency measurement and suction termination if fetal abnormalities are detected early in gestation. Although important, first trimester diagnosis of fetal abnormalities carries few disadvantages: difficulties in the technique of transvaginal fetal scanning, inability to detect all abnormalities, no pathological confirmation of diagnosis, significance of minor anomalies is unclear at present and there is a high spontaneous loss rate in fetuses with major malformations.

Increased nuchal translucency (NT) refers to a measurement above the 95th centile between 11 to 14 weeks gestation is a common phenotypic expression of chromosomal abnormalities including trisomy 21 and a wide range of fetal malformations, dysplasias, deformations, disruptions and genetic syndromes. Pathophysiology of increased NT can be explained by cardiac dysfunction, venous congestion in the head and neck, alteration in the extracellular matrix, lymphatic vessel hypoplasia,

anemia, hypoproteinemia and congenital infection. NT thickness can be combined with maternal age and serum biochemistry (free â HCG and PAPP-A) at 11 to 14 weeks to identify about 90% of affected fetuses. Enlarged nuchal translucency, absent nasal bone and abnormal Doppler waveforms in the ductus venosus are useful markers of trisomy 21 in the first trimester ultrasound screening, increasing the sensitivity of detection of affected fetuses to more than 95%, when combined with serum biochemistry (3).

Rottem (2) has proposed a classification of fetal anomalies based on their natural history: Class I include abnormalities that occur very early in pregnancy (anencephaly, spina bifida, conjoined twins and holoprosencephaly). Class II includes transient conditions due to accumulation of fluid in the lymphatic, urinary or central nervous systems i.e. increased NT, cystic hygromas, pleural or pericardial effusions, choroids plexus cyst or hydronephrosis. Their presence indicates an underlying chromosomal defect. Class III anomalies have a variable onset (diaphragmatic hernia, hydrocephalus) or are potentially unstable anomalies (exomphalos, enchephalocoele). Class IV is late onset anomalies (corpus callosum agenesis, microcephaly and duodenal atresia).

Detection of minor anomalies in the first trimester like choroid plexus cyst, mild renal pelvic dilatation, intra-abdominal cysts, umbilical cord cysts and echogenic foci in the fetal heart also affects second trimester screening. Many of these resolve by second trimester but their significance is unclear.

Detection rate of fetal anomalies using transabdominal scan as seen in the screening studies show a sensitivity of 13 to 14% whereas transvaginal scanning show as high as 32 to 65% in low risk populations and 57 to 74% in high risk populations.

Type of abnormality that can be detected using transvaginal scan in the first trimester include CNS anomalies (anencephaly, ventriculomegaly, holoprosencephaly, encephalocoele and Dandy Walker malformation), neck (cystic hygroma), cardiac (TOF, hypoplastic right and left heart, Ebstein's anomaly, VSD, ASD), renal (hydronephrosis, bladder outlet obstruction, renal agenesis), abdominal wall defects, hydrops, skeletal dysplasias and spina bifida.

To conclude, detection rate of first trimester fetal anomalies can be enhanced with experience in transvaginal scanning, good quality high frequency transducer and thorough knowledge of the embryological development of first trimester fetus. Since some anomalies are not evident in the first trimester, the 18 to 20 week scan remains the gold standard.

Now a days, 3D imaging to detect fetal anomalies is also emerging as a diagnostic modality during first trimester.

References:

1. Achiron R, Tadmor O. Screening for fetal anomaly during the first trimester of pregnancy: transvaginal versus transabdominal sonography. Ultrasound Obstet Gynecol 1999; 1.
2. Rottem S Ironfan: a new time-oriented malformation workup and classification of fetal anomalies. Ultrasound Obstet Gynecol 1997; 10: 373-4.
3. Nicolaides KH, Wegrzyn P. First trimester diagnosis of chromosomal defects. Ginekol Pol. 2005; 76(1):1-8.

Counseling and Prenatal Diagnosis for Mental Handicap

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It is generally accepted that Mental Retardation (MR) occurs in 2-3% of the general population. Most authorities discuss MR in two categories - 'Milder MR' (IQ 50-70) and "more severe MR" (IQ < 50). Prevalence of milder MR is 20-30/1000 and is seven to ten times more common than severe MR . Etiology of MR should be conceptualised as a specific diagnosis that can be translated into useful clinical information for the family including prognosis, recurrence risks, pre-ferred modes of available therapy and prevention by prenatal diagnosis. So the term etiology has a broad interpretation.

Genetic Counseling for MR includes

1) Comprehending the medical facts including the diagnosis, probable course of the disorder , and the available management
2) Appreciating the way heredity contributes to the disorder and the risk of recurrence in the specified relatives
3) Understanding the alternatives for dealing with the risk of recurrence
4) Choosing a course of action which seems appropriate to them in view of their risk , their family goals , and their ethical and religious standards and act in accordance with the decision
5) Helping the families to make the best possible adjustments to the disorder.

Risk of Recurrence will depend on the etiology. Important points to remember are :

1. Chromosomal causes of MR are seldom familial except in situations when one of the parent is a balanced translocation carrier. Risk of recurrence in donovo chromosomal disorders is low (usually < 1%). In translocation Down syndrome cases when one of the parents is a balanced translocation carrier the recurrence risk is variable. It varies from 2.5% if the father carriers a 14/21 translocation to 100% if either parent carries a 21/21 translocation.
2. In single gene disorders, the risk of recurrence will depend on the type of inheritance. In an autosomal recessive disorder risk of recurrence is 25% for the sibling. In autosomal dominant disorder the risk of recurrence for the sib is 50% if one of the parents is affected. If its a sporadic case (new mutation) risk of recurrence in sib is very low but gonadal mosaicism cannot be ruled out. In X-linked recessive disorders, risk of recurrence for boys is 50% where as females usually do not manifest.
3. Environmental causes, when identified, recurrence is unlike-ly unless the causative agent keeps on operating e.g. lead exposure, intrauterine exposure to teratogens.
4. In situations where a specific diagnosis is not reached emperic risk figures based on population based studies are used to counsel the family. Table IV gives emperic risk figures.

Prevention of Mental handicapp: Strategies at primary level can go a long way to prevent MR e.g. Iodine supplementation, adequate nutrition, prevention of anemia, avoidance of toxins like lead, avoid consanguinity, avoid very young and advanced age pregnancies, periconceptional folic acid, good antenatal, intranatal and perinatal care, screening for intrauterine infections, prenatal screening by maternal serum markers and routine immunization of children with measles and rubella vaccine.

Secondary Prevention includes , early diagnosis and treatment of curable illnesses (e.g. hypothyroidism, galactosemia, phenylicetonuria), coun-seling of parents with past history of affected children based on exact etiology / emperic risk figures, Prenatal screening using noninvasive biochemical and ultrasound markers and invasive prenatal diagnosis using Chorionic villus sampling, amniocentesis, cord blood sampling if exact etiology has been established (e.g. previous child affected with chromosomal anomalies, fragile X syndrome neurometabolic / neurodenenerative disorders etc).

Guidelines for TORCHES Tests and Its Interpretation

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The TORCH test, sometimes called TORCH panel, is a laboratory investigation to diagnose a group of chronic infections, all having common or similar clinical manifestations. These infections comprise of *t*oxoplasmosis, *o*ther infections, *r*ubella, *c*ytomegalovirus (CMV), and *h*erpes simplex virus (HSV). The "other infections" usually include syphilis, human parvovirus and coxsackie viruses. However, in recent years this group has further been extended to include Epstein-Barr virus, varicella-zoster virus, and hepatitis B virus. These infectious agents are grouped together because they can cause a cluster of symptomatic birth defects in newborns. This group of defects is usually similar or overlap with each other, hence sometimes called the TORCH syndrome.

These manifestations include small size for gestational-age, hepatosplenomegaly with or without

jaundice, thrombocytopenia, erythematous eruptions, CNS defects, including encephalitis, calcified deposits in the brain tissue with or without seizures, heart valve defects, and ocular anomalies.
The TORCH tests to diagnose and manage the infection in best possible way, should preferably be done in the first month of pregnancy or whenever the pregnant woman visits her obstetrician for the first time. However, problem arises when the patient comes later in the pregnancy or when the obstetrician prescribes the TORCH test at the late gestational period. The laboratory plays very crucial role in the diagnosis and confirmation of specific TORCH infection, as none of the clinical feature is diagnostic of specific infection, rather the infection is suspected on the basis of cluster of manifestations. At the laboratory level, the laboratory physician must convince herself or himself before communicating the report to the patient. If the laboratory results are not reliable, on the basis of false positive test, pregnancy can be terminated unnecessarily. But if the infection is misdiagnosed, a congenitally malformed baby is born and becomes a serious medical and social parental liability. All patients with IgG and IgM positive results should be counseled and retested using other confirmatory methods including IgG avidity for Toxoplasma, Rubella and CMV and nucleic acid amplification techniques such as PCR. The IgG avidity test is a specific method that helps in pin pointing the approximate time of exposure. The high IgG avidity test denotes the period of exposure of more than 4 months and it can save the precious pregnancy from termination. Single point IgM positive results should not be used for termination of the pregnancies, as these antibodies can be detected for several months after true infection and also the test can be positive falsely if a sub-standard test method is adopted. Beside the specific test methods, the nature of sample also becomes very crucial, therefore other fluids like amniotic fluid, cord blood can be used for confirmatory antenatal diagnosis of TORCH infections.
[* File contains invalid data | In-line.JPG *] Dr. Sarman Singh, has obtained his MBBS from KGMC, Lucknow and MD in Microbiology from PGIMER, Chandigarh. After MD he is working at the AIIMS for the last 18 years. He is well known clinical microbiologist and research scientists in the field with special interest in TORCH infections. He has published more than 200 research papers in national and international journals and more than 20 only on TORCH infections. He has also authored 1 book on Toxoplasmosis in India and another on TORCH infections. Dr. Singh is member of various national and international scientific bodies including American Society of Microbiologists, International AIDS Society and ISPAT.

Treatment of Herpes Simplex and Toxoplasmosis-Outcome

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Introduction: Toxoplasma and herpes infection are part of TORCH infections. If acquired during pregnancy they are capable of maternal to fetal transmission and can cause sequelae in the newborn.
Toxoplasmosis
Clinical Features: Most toxoplasma infections are self-limiting and treatment is not necessary. Fetal transmission has never been observed if the maternal infection has occurred before the pregnancy. However, primary infection during the pregnancy can result in congenital toxoplasmosis. There is strong effect of gestational age at maternal infection on transmission risk and earlier the gestation at infection, severe the sequelae.
Management Primary prevention is to follow simple hygienic measures such as avoiding contact with items contaminated with cat faeces.
For patients suspected of primary infection, the confirmation is by studying the IgG and IgM antibody levels, and IgG avidity studies. All patients with primary infection should receive spiramycin (3g/ day) from confirmation of infection till birth of the baby. These patients should be counseled for prenatal diagnosis of fetal infection by means of amniocentesis for polymerase chain reaction (PCR) for Toxoplasma DNA of amniotic fluid. In cases with proven fetal infection, termination may be required

if the ultrasound examination reveals severe fetal anomalies. Medical management – a three-week course of pyrimethamine and sulphadiazine with folinic acid alternating with spiramycin is prescribed. This combination is found to be more effective than spiramycin alone.
At birth, clinical and serological assessment of the newborn is done. Congenitally infected neonates should receive 1-year regimen of pyrimethamine, sulfadiazine and folinic acid. This is shown to significantly reduce neurological and ocular sequelae, especially in infants whose mothers had toxoplasma infection late in pregnancy.
Genital herpes simplex (HSV) is a common viral sexually transmitted infection in adolescents and adults and is acquired by mucocutaneous contact. The incidence of HSV infection is on the rise. The mortality in neonatal infection is reported as high as 25% and severe sequelae in a third of cases.
Clinical Features
Both HSV-1 and HSV-2 cause primary genital infection. Most of the vertical transmission takes place during asymptomatic infection. Nearly 5 – 10% of pregnant women have symptomatic recurrences and 25% in the last month of pregnancy
Treatment Primary Infection : Antiviral drugs are central to the therapy. They are shown to lower the frequency of recurrences but they do not eradicate the latent infection. These drugs are well tolerated with no drug related fetal abnormalities described as yet. In patients with frequent recurrences suppressive treatment is indicated. ACOG 1999 recommendations states that for women at or beyond 36 weeks of gestation with a first episode of HSV in current pregnancy, antiviral therapy should be considered (level B), and for women at or beyond 36 weeks of gestation who are at risk of recurrent infections antiviral therapy may not reduce the likelihood of cesarean delivery (level C).
Since 85% of neonatal herpes is near time of delivery the current practice is to do elective caesarean section (CS) for women with active or prodromal lesion at term. However, recently there is refocus on medical therapy (acyclovir) to decrease the HSV shedding and avoiding CS for this indication. CS is indicated if clinical herpes lesions and/or positive virus detection tests are present at the time of delivery. However, a history of genital herpes alone, in the absence of genital symptoms (active lesions or prodromal pain or burning) at the time of delivery or an episode of recurrent genital herpes in the weeks before the expected delivery date should not be taken as an indication for an elective CS section. After a clinical recurrence during pregnancy, vaginal delivery is possible, as soon all clinical lesions have disappeared.

Rubella – Time to Start a National Prevention Program

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Rubella (German measles) is a viral illness caused by a togavirus, genus Rubivirus (a single stranded RNA virus with a single serotype that does not cross react with other togaviruses), which was discovered by a German physician in the mid eighteenth century. In children it is a mild illness. However, if it occurs during pregnancy (specially the first half of pregnancy), it can cause fetal infection leading to miscarriages, still birth and birth of babies with Congenital Rubella Syndrome (CRS) in nearly 20% of the babies.
Congenital Rubella Syndrome is characterized by any or all of the following defects in the newborn.
i) Ocular – Cataract, glaucoma, pigmentary retinopathy, iris hypoplasia, cloudy cornea.
ii) Auditory – Sensorineural, deafness.
iii) Cardiovascular – patent ductus arteriosus, ventricular septal defects, pulmonary artery stenosis, myocarditis.
iv) CNS lesions – Microcephaly, psycho motor retardation, meningoencephalitis, behavioural disorders, speech disorders.
v) Miscellaneous – IUGR, hepatosplenomegaly.
Burden of CRS: 1,00,000 cases occur each year in developing countries. There is no statistical data from our country as diagnosis is not made; Mental retardation, congenital eye and ear defects are not

detected at birth and there is no central registration for such cases. However, we can extrapolate from the statistics available from USA (before vaccination was available), that in one epidemic in 1964-65, there were 12.5 million cases of rubella, over 2,000 cases of encephalitis, 11,250 abortions and over 20,000 cases of CRS, over 11,000 cases of deafness and 3,580 cases of blind children and 1,800 children with mental retardation were born.

All these handicaps burden the family, the society and the country. Caring for them takes up much more resources than a national vaccination program would entail. However, as is world evident, our planning has always been very short sighted.

Vaccination: The primary purpose of rubella vaccination is to prevent the occurrence of CRS. Two approaches are possible :

i) Prevention of CRS only, through immunization of adolescent girls and women of child bearing age.

ii) Elimination of rubella as well as CRS through universal vaccination of infants and young children (with / without mass campaigns), surveillance and assuring immunity in women of child bearing age.

Rubella vaccine was first used in late 1960s and is based on the live attenuated RA 27/3 strain of the virus. The are propagated in human diploid cells and have proven to be safe and efficacious. It is available in a monovalent form as rubella alone or as trivalent with measles, mumps and rubella together (MMR).

National Prevention Program: Rubella vaccination in national programs increased from 78 countries in 1996 to 123 by December 2002.

Best example of National Program is of USA where the following strategies are employed.

i) Universal dose of MMR at 12-15 months of age and 2nd dose at 4-6 years of age.

ii) Vaccination against MMR certificate essential for admission to school. If no documentation, revaccinate.

iii) A comprehensive health service checkup at age 11-12 years to reevaluate that immunity is present, otherwise revaccinate.

iv) All persons who work in health facilities should have documented rubella immunity.

v) Adults – all persons born after 1957 should receive at least one dose of MMR vaccine unless contraindicated.

vi) Women of child bearing age – MMR vaccine should be offered to all women of child bearing age who do not have acceptable evidence of rubella immunity whenever they make contact with the healthcare system. Vaccination of susceptible women of child bearing age should :

- Be part of routine general medical and gynecological out patient care.
- Take place in all family planning settings.
- Be provided routinely before discharge from any hospital, birthing center or other medical facility unless a specific contraindication exists.

Prenatal screening and postpartum vaccination: Prenatal serologic screening of women who have acceptable evidence of rubella immunity is generally not necessary but is indicated in all pregnant women, who lack acceptable evidence of rubella immunity. Upon completion of their pregnancy, vaccination with MMR before discharge from hospital / abortion clinic is must.

Colleges and other Post High School Educational Institutions: Risk of transmission is high at such places. College entry requirement for MMR vaccination certificate is a routine regulation.

International Travel : It is important to be protected with MMR before traveling to areas where these diseases are endemic. However, these are not essential documents checked before entry into USA.

Perinatal Hepatitis Infection and Its Prevention

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Hepatitis is one of the most common and contagious viral infection. Currently six types of hepatitis viruses have been identified: A,B,C,D,E. Each type of hepatitis has different implications for the

mother and her fetus.
Hepatitis A : produces no adverse effect on the fetus. It is not teratogenic, nor causes vertical transmission (except a few case reports). Transmission to the neonate from a acutely infected mother can occur via the fecal-oral route during delivery and postpartum. It appears reasonable to administer immunoglobulin to infants born to mothers who develop acute hepatitis A during third trimester or just before delivery, the cost-effectiveness of this procedure is not clear
Hepatitis B : In patients with acute hepatitis B, perinatal transmission occurs in upto 10% of neonates if infection occurs in first trimester and in 80-90% when infection occurs in third trimester. 10-20% women seropositive for HBsAg transmit the virus to neonate while those seropositive for both HBsAg and HBeAg transmit infection to approximately 90%. Chronic infection occurs in 90% of infected infants. The virus is not teratogenic, but low birth weight and preterm delivery may occur if acute infection occurs during pregnancy. All pregnant women should be tested for HBsAg at first antenatal visit. Infants of pregnant women with hepatitis B should receive H-BIG at birth and hepatitis B vaccine at birth, one month and 6 month of age.The risk of fetal hepatitis B infection is there after invasive prenatal diagnostic procedures. Delivery by cesarean section is not recommended to reduce vertical transmission. With appropriate immunoprophylaxis breast feeding poses no additional risk of transmission.
Hepatitis C: the rate of vertical transmission is 5%, higher in HIV positive mothers. Cesarean section is not recommended to reduce vertical transmission. Breastfeeding does not increase infection risk to infants. Immunoprophylaxis to neonate does not decrease the risk of vertical transmission.
Hepatitis D: perinatal transmission is reported, but this risk can be nil as immunoprophylaxis to neonate against hepatitis B is 100% protective.
Hepatitis E: acute hepatitis E infection during the third trimester of pregnancy not only results in vertical transmission of HEV but also results in significant morbidity and mortality to the neonate. Evidence of infection was found in 6/8 infants born to mothers with acute infection in third trimester, in a study from India. Two neonates developed hypothermia and hypoglycemia and died within 24hrs, massive hepatic necrosis was present in one. Four infants had hepatitis. There is no vaccine against HEVand Ig is not effective in preventing infection.

Routine Fetal Echocardiography: 4 Chamber View and Onthal Views – Is it Worth it?

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Cardiac anomalies constitute about 1% of all anomalies at birth. Most studies report an incidence of 6-8/1000 live births. Fetal incidence is more because of spontaneous abortions in a large number. An incidence of 30/1000 is seen in still births. Cardiac defects have a tendency to be associated with extra cardiac defects (25-40%) and with chromosomal anomalies in 15-25%.
Indications for fetal echo:
Based on findings
- Abnormal 4 chamber view
- Increased nuchal translucency at 11-14 weeks
- Hydrops
- Poly/Oligoamnios
- Arrhythmias
- Extra cardiac anomalies
- Twins – MA

Based on history
- Family history of CHD and/or syndromes
- Maternal : DM, collagen vascular diseases
- Teratogens : Rubella, alcohol, drugs

Detection rate has been increasing over the years. Studies in mid 1990s report a 33% detection on 4 chamber view; and a 66% detection on 4 chamber and outlet views. Study in 1993, Kirk et al of 3000 cases reports a 80% detection of structural anomalies.
Limitations:
4 chamber view will not allow evaluation of great vessels
- TGA, Fallot's, Truncue arteriosus, DORV
- Outlet VSD, over-riding of aorta
- AS, DS, Coarctation

4 chamber + outlet views will not detect
- Small VSD
- Mild AS/PS
- Mild Coarctation of aorta
- Secundum ASD

Fetal Cardiac Screening by Color Flow Mapping

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Antenatal Ultrasound evaluation of cardiac anatomy and blood flow is important as a part of routine screening anomaly scan as more than 90% of congenital cardiac defects occur in low risk pregnancies. Congenital heart disease affects between 1 and 8 per 1000 live births and 27 per 1000 stillbirths. The risk of congenital heart disease increases if one parent or prior child of the couple has had congenital heart disease. Risks are also increased with a variety of dominant or recessive genetic syndromes, and with aneuploidy. Approximately 5% of infants born with cardiac anomalies have a positive family history of congenital heart disease, and approximately 12% have abnormal chromosomes.
Fetal Echocardiography is indicated as a part of routine anomaly scan when there is maternal or family history of CHD, maternal diabetes, maternal drug exposure during pregnancy, maternal infection during pregnancy, maternal alcoholism and maternal connective tissue disorder. The fetal indications of Fetal Echocardiography are polyhydramnios, nonimmune hydrops, dysrrhythmias, extra cardiac anomalies, chromosomal aberrations and symmetric IUGR
Optimal time for Fetal Echocardiography is 18 - 26 weeks of gestation with high frequency (5-7 MHz) & high resolution ultrasound machine with color Doppler & Pulse Doppler function. It is not satisfactory at later gestation due to calcification of ribs & decrease in amniotic fluid. Adequate imaging of fetal heart is essential for accurate diagnosis along with proper training and expertise of sonographer. For good ultrasound imaging to screen fetal heart, it is desirable to resort to the echocardiography settings on the ultrasound machine which allows enhanced image contrast and tissue characterization. A faster frame rate improves cardiac imaging. Depth is minimized on display monitor and heart is scanned through an angle that avoids shadowing by fetal ribs and sternum. Enlarging the area of interest (zoom) allows visualization of details with in the heart. While adding color Doppler to gray scale image, high velocity scales are chosen for cardiac blood flow. The color Doppler is optimized by directing the angle of insonation of ultrasound beam parallel to the direction of blood flow and wall motion artifact is significantly reduced.
Color Doppler function adds significantly to our ability to diagnose Congenital Heart Disease and confirms the abnormalities suspected on grey scale scanning. It has special significance in evaluation of flow patterns across atrioventricular valves, the foramen ovale and semilunar valves. The presence of decreased or absent flow across the mitral and tricuspid valves during diastole, on Color Doppler suggests the presence of valvular stenosis or atresia. Color Doppler screening is also useful in confirming diagnosis of common atrio-ventricular valve and ventriculo-septal defect in four chamber view. It can study direction of flow in great vessels in three vessel view and also confirm diagnosis of

interrupted inferior vena cava or minimal to moderate amount of pericardial effusions.
Color Doppler is used to direct placement of the sample volume to obtain Doppler wave forms in Pulse Doppler function. Pulsed Doppler ultrasonography demonstrates the direction and characteristics of blood flow with in fetal heart and great vessels. To obtain accurate Doppler indices, the sample volume is placed distal to the respective valves and Doppler waveforms should be obtained during fetal apnoea.
Color coded Doppler flow mapping is essential part of fetal cardiac scanning. Besides indicating direction of flow and flow disturbances, it permits more rapid description of cardiac and vascular morphology.

Fetal Arrhythmia - Follow Up of the Intrauterine Condition

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"It is as natural to die as to be born, and to a little infant, perhaps, the one is as painful as the other"
Francis Bacon, Essays

Fetal arrhythmias are being recognized with increasing frequency over the last several years. Fetal echocardiography is very useful in the diagnosis and management of fetal arrhythmia. Fetal ECG can be constructed by combined use of two dimensional imaging and simultaneous M mode recording and matching the atrial and ventricular contractions. Approximately 1% of fetuses suffer from fetal arrhythmias. This figure may be an under estimation because most fetal arrhythmias are silent and resolve spontaneously. Fetal arrhythmias are best diagnosed by M mode and Doppler echocardiography and in fact they account for 15% referral for fetal echocardiography. Isolated extrasystole, both atrial and ventricular, are the commonest arrhythmia and are seen in nearly 90% cases of arrhythmias picked up on fetal echocardiography. These isolated ectopics without any structural heart disease carry benign prognosis and do not need any specific therapy. Important common arrhythmias are supraventricular tachycardia (most common), atrial flutter, atrial fibrillation, ventricular tachycardia and complete heart block.
Supraventricular Tachycardia: This is the commonest fetal arrhythmia if isolated extrasystoles are excluded. The heart rates are fast, usually beyond 180 beats per minute. [16] In most cases there is no underlying cardiac lesion; Ebstein's anomaly of the tricuspid valve and rhabdomyomas may be responsible in a small proportion of cases. Supraventricular tachycardia is generally well tolerated for short periods, but if it continues, it can cause myocardial dysfunction, atriventricular valve regurgitation and fetal heart failure seen as hydrops. Fetal hydrops manifests with the presence of ascites, pericardial and pleural effusions and skin edema especially in the scalp. Hydrops is seen in 40%-57% cases of fetal tachyarrhythmias. If supraventricular tachycardia has not been picked up clinically, fetal hydrops may be the first manifestation. All cases of tachyarrhythmias diagnosed in fetus should be serially followed to observe development of hydrops because it is a marker of poor outcome and inadequate response to antiarrhythmic therapy. Echocardiography can detect each of these findings of hydrops fetalis and hence is very useful. Electrophysiological studies in immediate post-natal period have revealed that atrioventricular re-entrant tachycardia over an accessory pathway is the mechanism in over 90% cases while atrioventricular nodal reentry and intra-atrial re-entry are the mechanism in the remaining. Electrophysiological mechanism of arrhythmia does not necessarily correlate with the outcome.
Treatment: Pharmacologic treatment for supraventricular tachycardia in fetus is indicated only in cases showing early features of hydrops and in whom a premature delivery is fraught with risk, either due to extreme prematurity or lung immaturity. Maternally administered drugs are the mainstay of therapy and therefore close monitoring of mother is essential. Drug, which have good fetal transfer,

are preferred. There are no randomized controlled trials comparing treatment with various drugs but certain trends have emerged. Digoxin remains the drug of choice for initiating therapy in fetal supraventricular tachycardia. Placental transfer of digoxin is good. Ratio of fetal to maternal digoxin level is one in most of the cases. Both fetus and mother tolerate much higher doses of digoxin. Intravenous/oral boluses of 0.25-0.5 mg are given till serum level of 1 or 2 ug/ml is attained. Digoxin absorption is impaired in pregnancy. This explains the greater requirement of digoxin by mother and fetus as compared to older children and adults. As drugs are used in high doses, mother should be carefully observed for any side effects although these are very uncommon. Daily maternal monitoring with ECG should be done. It is not easy to exclude the possibility of WPW syndrome in the fetus having supraventricular tachycardia, therefore use of digoxin may be hazardous as it may precipitate excessive ventricular rate during atrial fibrillation and endanger life. Though risk is small, it is better to explain this risk to the parents. Positive ionotropic effect of digoxin is helpful in reversing the ventricular dysfunction caused by tachycardiomyopathy apart from its effect in controlling the rate of tachyarrhythmia. It must be noted that presence of hydrops significantly impairs the efficacy of digoxin.

Other drug used in the treatment of supraventricular tachycardia includes propranolol, which can be added to digoxin. If there is no response then either Sotalol or flecainide can be used. If maternal drug therapy is unsuccessful, direct umbilical vein or fetal intramuscular drug administration can be considered. If there is no response to drugs, a premature delivery may have to be performed. Echocardiography again helps in follow up of these cases to monitor response to therapy.

Complete Heart Block: A persistent fetal heart rate of less than 100 beats per minute is defined as fetal bradycardia. Persistent bradycardia should raise suspicion of complete heart block which is the commonest cause of prolonged fetal bradycardia. Uncommonly fetal bradycardia can be the result of blocked premature atrial beats. Echocardiography can distinguish complete heart block from sinus bradycardia by showing a normal atrial rate, a slow ventricular rate and presence of atrio-ventricular dissociation. Complete heart block can be an isolated abnormality, in which case the mother often has a connective tissue disorder like systemic lupus erythematosis, which may be subclinical. In this setting the heart block is caused by circulating antibodies, especially anti Ro and anti La antibody. These antibodies cross the placenta and damage the fetal conduction tissue producing complete heart block. The bradycardia is generally well tolerated but a small number of fetuses decompensate producing intrauterine heart failure. Maternal treatment with sympathomimetic drugs such as salbutamol and/ or steroids may be beneficial. Intrauterine pacing has been tried but has not been successful. Alternatively an elective early delivery with postnatal epicardial pacing can be performed if fetal compromise is evident.

In another half of cases with complete heart block, there is an underlying cardiac malformation such as corrected transposition of great arteries, atrio-ventricular septal defect, heterotaxy syndrome etc. The outcome of these cases depends upon the underlying cardiac defect. Generally the mortality is high in this group with intrauterine deaths.

Management and Counseling for Fetal Cardiac Disease

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The Exciting Future of Prenatal Diagnosis in India

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The desire of having a normal baby is universal. However, in India due to the tremendous socio-economic burden of looking after a child with a genetic disorder or disability the parents readily accept prenatal diagnosis to ensure birth of a normal baby. These are exciting times as the newer technologies of prenatal diagnosis have been established in a number of centers in India, and are available to the patients at an affordable cost. This is a great boon, as the tests when performed in the West are prohibitively expensive, so that they can be used only by a handful of Indian patients.

I present a status report on the situation in India, drawing on personal practice at our center. The superior quality of ultrasound equipment has been the most important step in improving and expanding the scope of prenatal diagnosis in India. In all cities there are some very good ultrasonologists equal to the best in the world. However, we have to make sure that the quality of ultrasounds as commonly performed for the majority of women should be of acceptable quality. To achieve this we have to introduce immediately certification and accreditation for the ultrasonologists.

Chromosomal disorders are common in India as in other countries. To prevent these disorders many countries have introduced large-scale biochemical screening, and in those cases where the results indicate high risk, follow this up with chorionic villus sampling or amniocentesis for confirmation. This approach is applicable to a few in India, due to considerations of cost. The way to go in India may be careful ultrasounds in the first or the second trimester, although in the cities the demand and uptake of double, triple or quadruple test is increasing, and many centers are able to offer amniotic cell cultures if required. FISH analysis in those cases requiring rapid diagnosis is available in India. QF PCR needs to be set up to diagnose aneuploidy because it is accurate, easy and cheap. However one has to select the cases where one may rely only on FISH or QF-PCR studies, because numerical abnormalities of chromosomes other than the five commonly tested and structural abnormalities remain undiagnosed by this method.

The biggest advances have been made in the application of molecular methods for prenatal diagnosis of a large number of Mendelian gene disorders. For example, at Sir Ganga Ram Hospital we have carried prenatal diagnosis of almost 1000 cases of beta thalassemia, and about 300 cases of Duchenne muscular dystrophy and spinal muscular atrophy, fragile X syndrome, hemophilia A and B, intra-uterine infections by AF-PCR (70), oculocutaneous albinism, congenital adrenal hyperplasia, Wilson disease, deafness due to connexin 26 gene mutations, and familial hypercholeterolemia. In fact in can be said that for any disorder for which the gene is known and cloned, prenatal diagnosis is possible. The obstetricians often do not appreciate that the affected child needs to be studied first to identify the mutation causing the disorder, and then only can one proceed to prenatal diagnosis. Secondly, it should be recognized that often multiple genes might cause a particular phenotype, so that time is required to first find out the gene involved in a particular case.

Woefully the facilities for prenatal diagnosis of lysosomal and other biochemical disorders are very poor and this problem needs to be addressed. A network of genetic centers is being established along with creating a website (geneticsindia.com) listing all the tests that are being done in India, and the laboratories offering them with their contact details. These facilities will go a long way to help the many patients in India.

Fetal Cells and Nucleic Acids in Maternal Circulation: Finally Useful for Non-invasive PND and Explanation of Maternal Diseases

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An intense effort has been undertaken to develop a non-invasive alternative for prenatal diagnosis,

based on the enrichment of fetal cells and now free DNA from the maternal circulation. Regadingly our laboratory pioneered magnetic cell sorting (MACS) for the enrichment of fetal erythroblasts, which enables us to identify fetal aneuploidies.
Prompted by such reports, the NIH conducted a large clinical study, with the aim to determine the efficacy of a fetal cell based approach for the detection of fetal aneuploidies. The study also examined the merits of different fetal cell enrichment approaches. The results of this NIFTY study indicated that fetal cells can indeed be detected in a large number of pregnancies. Although MACS based approaches appear to be more effective than methods using flow sorting, the resulting sensitivity and specificity are not yet at the level required for clinical applications. Furthermore recent work demonstrated a large proportion of fetal erythroblasts displaying an apoptotic phenotype, which may influence the analysis of these cells by FISH and PCR.
Now the non-invasive diagnosis of the Rhesus factor and other single gene problems has been achieved using PCR.
Future work will focus on reliable, simple enrichment procedures with minimal fetal cell loss, automated fetal cell recognition and optimisation of the tools for the analysis of these rare cells. As a "spin-off" of this research it has become clear that the microchimerism of fetal cells in the ciculation of pregnant women can have negative consequences on the mothers , e.g. that y-containing cells from previous pregnancies with a boy where identified in the periphery and skin lesions of women with sclerodermia or polymorphic eruptions of pregnancy.
Regarding women with Hashimoto thyreoiditis it was found that fetal cells can be identified in the thyroid gland looking like their surrounding-tissue thus indicating their pluripotency.
Our lab found an association between an increased amount of fetal DNA in women with preeclampsia and HELLP Syndrome, indicating the influx of haploidentical fetal DNA into the maternal circulation as the "toxin" of gestosis. We also found that increased fetal DNA preceeds preeclampsia manifestations already in the second trimester of pregnancy offering a new predictive test.

Preimplantaion Genetic Diagnosis

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Maternal Serum Screening for Genetics Disorders Maternal and Foetal Perspective

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Collectively genetic disorders and birth defects are encountered in more than 2% of term pregnancies, but individual genetic disorders occur at a much lower incidence. Hence we can not use diagnostic tests for such disorders on the entire population. Concept of screening programs for genetic disorders is aimed to identify the very small high risk sub-population from the entire, low risk, population so as to increase the detection rates in a cost-effective and safer manner. Requirement of a good genetic screening program are,
• The target disorder should be well defined, have significant incidence and severity to affect the population.
• Screening test should be simple, safe (preferably non-invasive), affordable, easily available and with acceptable false positivity & sensitivity.
• Availability of accurate diagnostic test to confirm the diagnosis in screen positive individuals.
• Follow-up management options should be there for the high risk population identified by the screening test.

Very few screening programs can meet all these requirements. NTD, Down's syndrome, Thalassemia, Cystic fibrosis are some of the popular maternal biochemical screening programs. Large, over-populated country like India faces peculiar problems in implementing screening programs

• Very large population, lack of awareness, late antenatal registration (after 5-6 months), superstitions/ myths.
• Genetic screening not mandatory even in high risk pregnancies, lack of national policy.
• Cost factor: 40% deliveries conducted in public sector, where totally free services are provided. The meagre funds are not adequate to cover even higher priorities like malnutrition, prematurity, infectious diseases.
• 60% deliveries are conducted by private sector, which is self-funded by the patients as insurance coverage is still very low.
• Low demand leads to higher cost, which prevents the extensive coverage of population.
• Lack of standard protocols for laboratory assays, interpretation & reporting.
• Limitations of genetic screening programs: reduced positive predictive value, false positivity confusing risk assessment, ethical issues.
• Paucity of well equipped genetic laboratories for confirmatory tests, high costs of these tests.

Main points for consideration in evaluating any screening program are Detection Rate (DR), false positivity, false negativity and correlation of these with actual diagnostic tests. Performance of various screening tests for NTD and Down's syndrome will be elaborated in the talk.

Sample survey data from 12 centres in different parts of the country will be also presented in the talk. It is highly essential to develop a national policy for screening programs for selected target disorders so as to down-stage these diseases.

Cord Blood Collection for Stem Cell Transplant

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Until recently, blood that remained in the umbilical cord and placenta after delivery was routinely discarded. Now that this blood is known to contain both haematopoietic stem cells (HSC) and pleuripotent mesenchymal cells (PMC), there has been a substantial increase in the clinical use and research investigation of umbilical cord blood in haematopoietic transplantation and regenerative medicine. The otherwise discarded 80-100ml of cord blood can reconstitute the haemopoitic system in patients usually children and lately for transplantation in adults.

Almost 16 years after the first successful umbilical cord blood (UCB) transplant, more than 6,000 haematopoietic transplants have been undertaken worldwide for indications including thalassemias, sickle cell disorders, haematological malignancies, immune deficiencies and metabolic disorders.

The use of stem cells from cord blood has several clear advantages over bone marrow donation or collection of peripheral stem cells from a donor. These include :

· Limitless supply
· Available on short notice for transplant
· No donor attrition compared with bone marrow registry
· Ethnic diversity easier to achieve
· Painless collection of stem cells
· Higher proliferative capacity
· Lower rate of acute grafts vs host disease

However cord blood for stem cell transplant has certain disadvantages which include :

· Inability to obtain additional 'donor cells' for second transplant
· Fewer total haemopoietic progenitor cells due to small volume
· Slower engraftment

All consents for cord blood donations must be optimally obtained from women before labour and preferably in third trimester. It is important to screen donors prior for genetic and infectious disease.

Cord blood is usually not collected from pregnancies less than 34 weeks, multiple pregnancies, group B streptococcus carrier, prolonged rupture of membranes, chorioamnionitis, meconium stained or malodorous placenta. The acquisition of cord blood units involves the consent process cord blood collection, processing of blood, cryopreservation and storage.
Cord blood collection at delivery can be either 'in vivo' (while the placenta still remains in utero) or 'in vitro' in a specialised apparatus. Obstetric providers should undergo training on proper technique of cord blood collection especially for 'in vitro' technique. Several perinatal factors have been associated with increased nucleated cell counts in cord blood units. Higher cell counts are associated with first born infants, increased birth weight, prolonged labour, increasing gestational age, shortened interval to cord clamping.
It is mandatory to screen collected samples and maternal blood using the same tests as those applied to blood donors, which includes HIV-1 and 2, anti HCV, hepatitis B surface antigen and TPHA for syphilis. Most cord blood bank would also screen for anti CMV. All blanked units are eventually typed for HLA-A, B and DR. Full blood counts are done on all cord blood collection prior to processing.
Cord blood donations are altruistic, directed donations in 'at risk families', or donations in 'low risk families'. Altruistic donations provide feasible source of HSC when compatible bone marrow donors cannot be found either in the family or from one of the six millions bone marrow donors worldwide. Directed collection and storage in "at risk" families is currently recommended for siblings when there is a known genetic disease amenable to HSC transplant. Public cord blood banks are currently providing cord blood collection and storage for altruistic and at risk donations.
Cord blood donations directed "in low-risk" pregnancies is based on presumption of a child developing a disease in later years that can be treated by this stem cells stored at birth. Most private cord blood banks are promoting autologous donation in low risk pregnancies.
Umbilical cord blood represents an exciting new sources of HSC. The obstetrician represent the first line of information and counseling to pregnant women on pros and cons of cord blood collection and banking. It seems that in future UB stem cells may be the material of choice in surgical transplantology, immunotherapy and gene therapy.

Genetic Aspects of Recurrent Spontaneous Abortions

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Recurrent pregnancy losses such as spontaneous abortions, stillbirths and early neonatal deaths are tragic events for both the mother and the family. Recurrent miscarriage is defined as the occurrence of three consecutive first-trimester losses of pregnancy and affects 1% of women. The purposed causes of recurrent miscarriage include, endocrine disorders, anatomical causes, immune factors, thrombophilia, and genetic causes. Genetic causes include chromosomal anomalies either structural rearrangements or aneuploidies , various single gene disorders especially X linked Dominant disorders and various hypercoagulable states due to mutations in factor V , prothrombin gene and MTHFR genes. However despite extensive investigations cause remains unknown in 50% of cases. Presently,the only recommended investigations are testing for lupus anticoagulant and anticardiolipin antibody levels (to diagnose antiphospholipid syndrome, an acquired thrombophilia) and the karyotyping of both parents for chromosomal abnormalities. Women with antiphospholipid syndrome should be offered treatment with aspirin and low molecular weight heparin. Couples with chromosomal abnormalities should be referred to a clinical geneticist with whom the options of prenatal diagnosis, pre-implantation genetic diagnosis, donor gametes and adoption in subsequent pregnancies should be discussed. Siblings of carriers of chromosomal rearrangements should be offered chromosomal analysis and counseling. Couples with unexplained recurrent miscarriage should be offered appropriate emotional support and reassurance that they have a good prognosis for future pregnancies.

Recurrent Pregnancy Loss- Maternal Management

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Hydrostatic and Osmotic Pressure Gradients Produce Manifestations of Fetofetal Transfusion Syndrome in a Computerised Model of Monochorial Twin Pregnancy

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Objective: Despite recent advances in the assessment and treatment of feto-fetal transfusion syndrome (FFTS), its underlying mechanism remains controversial. We aimed to determine if the clinical features of FFTS could be explained by uni- or bi-directional inter-twin transfusion along placental vascular anastomoses.
Study Design: We constructed a dynamic computerised model of monochorial twin feto-placental units based on numerous interrelated haemodynamic, osmotic and metabolic physiologic variables. The circulations were then linked by various combinations of direction and number of arteriovenous anastomoses.
Results: With unidirectional anastomoses, disease severity, characterised by disparity in blood solids, depended on donor arterial pressure but not the number of anastomoses. In the chronic state water movement resulting from raised osmotic pressure in the recipient and reduction in the donor, produced hydro-osmotic pressure equilibrium, reducing anastomotic flow to near zero. ANP driven urine production was markedly increased in the recipient due to the raised vascular hydrostatic pressure component.
With bi-directional anastomoses, recirculation between twins reduced discordancy in colloids and hæmatocrit, and the clinical picture was determined by the degree of asymmetry in the number of connections.
Conclusions: Severe manifestations of FFTS can be explained by unidirectional inter-twin transfusion, with lesser degrees by asymmetric bi-directional transfusion.

Prenatal Diagnosis in Multiple Pregnancy

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The issues surrounding prenatal diagnosis in multiple pregnancy are complex. Accurate determination of chorionicity is of vital importance and failure to determine this should trigger consideration for referral to a specialist. The choice of screening methods for detection of chromosomal abnormality is limited, but existing data demonstrates the advantages of nuchal translucency screening. The possibility of obtaining discordant results and options for management should be discussed in advance. Invasive tests are technically more difficult and associated with a higher risk of procedure related pregnancy loss. Repeat invasive testing is needed more often than that for singleton pregnancy. Selective termination is technically feasible in both mono and dichorionic pregnancies, although the risks are higher with the former. It is likely to be more acceptable than high-order multifetal reduction performed in the absence of fetal abnormality.

Ultrasound in Multiple Pregnancy and for Specific Anomalies

Ratna Talukdar

Prevalence and Significance of Cranial Scan Abnormalities in Preterm Twins in Relation to Chorionicity and Discordant Birth Weight

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We studied 85 monochorionic (MC) and 94 dichorionic (DC) twin pregnancies (341 infants) delivered between 24-34 weeks of gestation to determine the incidence of cranial ultrasonographic lesions and its neurological significance in relation to chorionicity, twin-twin transfusion syndrome (TTTS), and discordant birth weight.
The cerebral lesion was seen in 14% of preterm twins with MC infants having a 7 fold higher risk than those of DC twins (95% CI 3.28 to 15.8). In MC group, infants with discordant weight (37%), TTTS (38%), single intrauterine death (67%) had higher incidence of cerebral lesions than those with concordant weight (7%). Similarly, incidence of cranial scan abnormality was higher in discordant DC compared with concordant weight infants (13% vs 2%; $P<0.05$).
Cerebral palsy (8% vs 1%; $P<0.05$) and neurological morbidity (15% vs 3%; $P<0.05$) were higher in MC than DC infants. In MC twins, neurological disability was higher in infants with discordant birth weight (42%; $P<0.01$), TTTS (37%; $P<0.01$) and co-twin demise (60%; $P<0.01$) than those with concordant birth weight (8%). But incidence of cerebral palsy was higher in discordant than TTTS infants (19% vs 4%; $P<0.05$). Similarly, discordant DC infants had higher neuro-morbidity than the concordant group (5% vs 1%; $P<0.05$). Irrespective of chorionicity, neurological morbidity was higher in the concordant (57% vs 3%; $P<0.001$) and discordant infants (73% vs 4%; $P<0.001$) with cranial scan lesions than those with normal cranial scan.
In conclusion, preterm MC infants were more susceptible to cranial injury and neurological morbidity than gestation age matched DC twins. Discordant birth weight, TTTS and survivors of co-twin demise were independent risk factors for cerebral white matter lesions and neurological morbidity.

Maternal and Fetal Medicine at Primary Care Obstetrics and Rural Health Care

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In most primary health care level, obstetrics is still accompanied by major casualties. This is mainly because of limitations in health services, certain diseases which are common in rural areas and also because of many adverse social factors. The great killers at this level are same everywhere in world - eclampsia, anaemia, haemorrhage, difficult labour & sepsis. Though many of these problems are unpredictable, many can be prevented by advances in fetal & maternal medicine. It is important to understand the problems at this level which are definitely different than urban sections. Early marriages & childbearing, consanguinity, poor nutrition & more contracted pelvis & uncontrolled infection need to be tackled on medical as well as social grounds. Another important issue, which is very common, is high parity & its consequences. At many centers, though modern medical facilities are available, people don't have faith in these facilities, doctors & hospitals. Good counseling & lot of public awareness is needed in this situation. All this is indirectly related to economic development &

education. We need to focus upon many complications lead by quacks as well as by birth attendants. It is worthwhile to slowly replace them by midwives & skilled birth attendants. While advocating this, it is very essential to study the local situation & social attitudes to gain confidence to use all modern facilities, which should be result oriented & last & very important is good communication with patients. There are many such examples that few benefited people do come forward for our rescue whom we should encourage to form support groups. These groups will help us in percolating all advances at primary health care level. We should never aim for full specialist care at primary level but try to fulfill all parameters of basic maternity services. Additionally we should not hesitate in offering choices of all modern advances in given situation. We can keep option of accepting it or not to rural people. All these efforts would definitely help to make childbirth safer at this level & will help to bring down our very high maternal & perinatal mortality & morbidity.

4D US in Prenatal Diagnosis

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Antenatal MRI

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Magnetic resonance imaging (MRI) was first used in prenatal diagnosis in the early 1980's. It became obvious that, MRI allowed for greater resolution of the fetus as compared to the commonly used ultrasound. MRI is the ideal choice because of lack of ionizing effect, multiplanar capability and inherent soft tissue contrast. Although seen as a promising technique, fetal MR studies were limited by long image acquisition times, that necessitated sedation of the otherwise moving fetus. Technical advances of the early 1990's produced fast MR techniques, better coils and gradients. This paved the way for meaningful imaging of a fetus without the need for sedation. Single-shot fast spin echo (ssFSE) sequences provide evaluation of the fetal organs, especially of the brain in a detailed fashion. No maternal sedation or fetal curarization is required with the newer scanners. The entire gravid uterus is first scanned in three planes orthogonal to the mother. Subsequent planning is performed to obtain planes orthogonal through the fetal organ of interest. Each sequence is then used a scout for the subsequent sequence, depending on the indication and the target organ. The scan time per sequence ranges between 15 to 23 sec. The whole study takes about 15 to 20 minutes depending on the indication and fetal movements. The sequence can be repeated in cases where fetal motion causes artifacts. 3D reconstructions can also be obtained.

The MRI study is usually well tolerated by all patients. There is no known harm to the fetus. However it is avoided in the first trimester which covers the period of organogenesis.

Ultrasound is a useful tool in screening an unborn child for birth defects. But sometimes, it reveals only a limited amount of information. This is due to inadequate visualisation of specific fetal anomalies as well as fetal position, maternal body habitus and the amount of liquor. Before MRI, parents who were provided with findings of questionable significance after fetal ultrasound, had no option for further investigating the situation. In difficult cases, MRI is an option that adds to the ultrasound diagnosis to delineate the abnormality better. Another frontier in which MRI has proved invaluable, is in cases where additional information can improve prenatal parental counselling and provide clearer options to the parents to continue or discontinue the pregancy. It also provides reliable information in multifetal pregnancies. It has a role in evaluation of the umbilical cord and the placenta. The uterine wall and the adnexal regions are better evaluated due to the wider field – of- view available on the newer scanners.

Also, there are avenues yet to be explored in fetal medicine and MRI may be a significant tool in future

research. Estimation of fetal weight using ssFSE sequences, has been reported by the American Roentgen Ray Society, as recently as May 2003. An attempt is being made to assess lung maturity depending on the intensity of the liquor on T2 weighted images. MR spectroscopy of the amniotic fluid may be helpful in assessing fetal renal function. MR spectroscopy of the fetal brain is a potential and powerful research method and could eventually be a useful clinical tool for the evaluation of in-utero brain metabolism.
Thus, although obstetric imaging will continue to be dominated by ultrasound because of it's easy availability, low cost and easier performance, MRI has created a special niche for itself with definite indications. It is evolving as a complementary investigation to ultrasound and has a vital role when in–utero or immediate post-delivery treatment is an option. However, high cost continues to be a limiting factor for wider usage.

In Utero Fetal Medical Therapy

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The concept of the fetus as a patient has evolved over time with increasing knowledge of fetal development, pathophysiology and diagnosis of disorders in the prenatal period. Treatment of the fetus in some instances may be advantageous over treatment of the newborn due to unique aspects of fetal development and biological characteristics of fetal development.
Medical therapy in the fetus is possible by administration of pharmacological agents either to the mother or directly within the fetal compartment. Many disorders have been successfully treated with fetal therapy.
Rh isoimmunization was the first example of successful treatment of fetal medical disorders in utero and serves as the model for the management of other disorders.
Corticosteroids have been used for augmentation of fetal lung maturation for over thirty years. Published data supports the use of dexamethasone or betamethasone for the antenatal management of preterm labour. This decreases the incidence of RDS and intracerebral hemorrhage in the neonate. The mechanism of corticosteroid action is probably by induction of the fetal pulmonary enzyme complex and enhancement of surfactant synthesis.
Fetal infections acquired in utero can be the cause of much morbidity & mortality and treatment in the neonate may not be effective. Some of the infectious diseases which can be prevented by in utero vaccination are Pertussis, Tetanus, Rabies, Meningococcal and Hepatitis B & CMV. A live attenuated CMV vaccine is undergoing preclinical & clinical trials to evaluate the efficacy to decrease incidence of infection. Various drug regimens are in use for the prevention of fetal toxoplasmosis. The efficacy of perigestational therapy to prevent maternal - infant transmission of Human Immunodeficiency virus is unequivocal. Recent data suggests the use of aggressive multi drug therapy to eliminate the risk of transmission.
Congenital adrenal hyperplasia due to 21 hydroxylase & 11b hydroxylase deficiency are treated by the in utero suppression of the adrenal axis in the female fetus. In utero therapy for the management of fetal thyroid disorders is controversial.
Fetal arrhythmias resulting in fetal hydrops have a mortality of 50-98%. Treatment of sustained fetal arrhythmias with antiarrhythmic drugs is well known, Advances in imaging, newer drugs and techniques of fetal administration of medicines have vastly improved the clinician's ability to diagnose and manage fetal rhythm anomalies.
The efficacy of periconceptional folic acid supplementation to reduce the risk of neural tube defects and their recurrence in families is well established.
Future directions for in utero fetal therapy lies in improving fetal access and improved technology to target successful fetal gene therapy.

First and Second Trimester Biochemical Screening

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3D and 4D Ultrasonography for Genetic Syndromes- Beyond the Fetal Portrait

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The practice of fetal medicine has been witness to revolutions in neonatal anesthesia, neonatal surgical technique and novel methods of *in utero* surgery. These factors combined with a pressing social and legal need for informed parental counseling, have placed new responsibilities on the shoulders of the ultrasonologist involved in the care of abnormal pregnancies. Advances in transducer technology and computer software are changing the way ultrasound scans are performed today and three dimensional (3D) and real time three dimensional (4D) technology is being used extensively to gain and display anatomical and hemodynamic information. This treatise reviews the application of these newer techniques in enhancing the data that can be obtained by ultrasonography.

3D ultrasound first became popular because of its ability to display the fetal face in a surface rendering mode. Currently available equipment has gone a long way beyond this, and permits the automatic acquisition of volume data sets which can then be processed for display in various modes. Volume acquisition can be done using a static 3D method or a 4D real time method. Color Doppler and Power Doppler flow information from the entire volume of tissue being imaged can be acquired at the same time. Once the data has been acquired, it can be analysed, processed and displayed using various software capabilities that facilitate obtaining optimal information. The specific advantage of a 3D/4D data set is that cross-sectional views can be obtained in any plane, at any orientation, in any direction and at any depth. It is possible to scroll through the entire volume using buttons, keys and track balls on the control panel. The display can be made in various ways: in a single plane, of a multiplanar type with a simultaneous view of three orthogonal planes, a tomographic multislice type akin to CT and MRI, or, a rendering of the data in various modes. These rendering modes include surface, minimum, inversion and glass body modes. In a multiplanar display, three planes are displayed perpendicular to each other. Since any such three planes intersect at a point it is possible to navigate through the volume by moving the point. Tomographic multislice display is a newer volume technique that displays cross sections parallel to each other, at any selected slice thickness in any selected plane orientation. After acquiring a volume, instead of scrolling through the volume, a series of slices can be displayed in any selected data set orientation. Information can be displayed as a grey scale for structure, in color for vascular morphology or as a combination of grey scale structure and color vascular morphology information. Volume Contrast Imaging is another new 4D technique that can enhance information obtained.

The sensitivity and specificity of prenatal diagnosis of fetal malformation has improved due to these technologic advances and innovations. 3D and 4D techniques have been central to this revolution and should be used liberally to enhance the availability of relevant information to the practicing specialist of fetal medicine.

This presentation reviews the application of these newer techniques in enhancing the data that can be obtained by ultrasonography.

Invasive Prenatal Diagnosis: Whats new?

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Stem Cells from Cord Blood and for *In-Utero* Therapy

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Umbilical cord blood is rich in hematopoietic stem cells. At birth, it can be collected, HLA-typed and stored. Cord blood is successfully used since over 10 years as source of transplantation of hematopoietic stem cells, in addition to bone marrow and mobilized peripheral blood stem cells. Allogeneic transplantations are performed between HLA-identical siblings and from HLA-matched unrelated donors. Most recipients of cord blood are children with leukemia or genetic disorders, but also increasingly adolescents and adults. Based on the promising results, cord blood banks with cryopreserved, HLA-typed cord blood samples from anonymous donors are set up worldwide, ready to be used as allogeneic stem cell graft. Additionally, so-called "private" cord blood banks were set up, providing the possibility to store cord blood at birth from healthy children with no affected family member for a possible autologous stem cell transplantation in the future if the child later develops a disease such as leukemia. To date, there is no established indication for an autologous cord blood transplantation. Nevertheless, the plasticity and multipotency of adult stem cells, which has been discovered recently, could lead to a possible autologous use of cord blood stem cells for different indications in regenerative medicines (cell- and organ replacement / regeneration). So far, however, this remains speculative.
Prenatal in-utero stem cell transplantation is promising therapeutic option for genetic disorders, which is now at the edge of moving from preclinical research into clinical application. The first clinical experience shows that some form of severe immunodeficiency can be treated successfully in-utero. No therapeutic success has been achieved in genetic disorders which do not severely affect the immune system, due to immunologic rejection and hematopoietic competition between donor and host cells. Therefore, new strategies are being developed, including graft modification, prenatal conditioning of the fetus, postnatal re-transplantation after prenatal induction of immune tolerance, and fetal gene therapy using autologous fetal stem cells. The use of non-hematopoietic (e.g. mesenchymal) or pluripotent stem cells will probably lead to an expansion of the spectrum of indications. Simultaneously, ethical implications, in particular regarding fetal gene therapy and the use of pluripotent stem cells must be addressed.

Endoscopic Surgery in the Smallest Patient

Shailesh Kumar

Prevention Therapy of Rh Disease

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In the late 1960s clinical trials showed that the incidence of Rh D immunisation of Rh negative women could be dramatically reduced by the administration of Rh D immunoglobulin (Ig) after delivery. In 1968 Australia became the first country to become self sufficient in Rh D Ig with the introduction of the Rh program which recruited donors for plasma collection for the production of Ig. Studies subsequently showed that immunisation rates could be further reduced by prophylactic administration of RhD Ig during pregnancy.
The administration of RhD Ig to non immunised Rh negative women for antenatal sensitising events,

for antenatal prophylaxis at 28 and 34 weeks gestation and postnatally where the infant is Rh D positive is now well established in clinical practice. This has been achieved through the development of Clinical Practice Guidelines on the use of Rh D Ig by the National Health and Medical Research Council (NHMRC), and support and endorsement of these guidelines by the Royal Australian and New Zealand College of Obstetricians and Gynaecologists (RANZCOG). The Australian Red Cross Blood Service and the Australian plasma fractionator CSL Bioplasma have developed and widely distributed both professional and consumer information which have raised awareness of this important preventive strategy.

The prophylactic use of Rh D Ig has led to a significant reduction in the incidence of haemolytic disease of the newborn which now occurs only in 1 in 1000 live births. Although now a rare pregnancy disorder, Rh isoimmunisation continues to be a challenging obstetric complication. While the face of the disease has undergone many changes in recent years, the principles of management remain unchanged. Pregnancies at risk are identified by past obstetric history and by the antenatal detection of red cell antibodies with serial measurement of antibody levels. In cases of paternal heterozygosity, the real risk of fetal disease can be determined using DNA techniques to determine the fetal blood cell type with tissue obtained from amniocentesis and now by molecular analysis of circulating cell free fetal DNA in maternal plasma.

Sensitised pregnancies are monitored for the development of fetal anaemia. Since its introduction into clinical practice by Liley over 40 years ago, spectral analysis of amniotic fluid bilirubin as an indirect measurement of fetal anaemia has been the primary method of screening for fetal anaemia. Noninvasive evaluation is now possible with the use of doppler ultrasound to detect changes to fetal blood flow secondary to anaemia, most commonly using the middle cerebral artery, and ultrasound evaluation of fetal haemopoietic organs and for the diagnosis of hydrops. Ultrasound guided intrauterine transfusion of red blood cells remains the treatment for the severely affected fetus. Perinatal survival rates in the non hydropic fetus are reported at over 90% and long term studies have revealed normal neurological outcomes for more than 90% of cases.

Prevention of Rh D isoimmunisation remains one of the triumphs of twentieth century medical practice. Over a few decades an understanding of the scientific basis and pathophysiology of haemolytic disease was gained, and an effective preventive strategy developed. The remaining challenges in prevention are maintaining awareness of a now rare disease, continued widespread adoption of appropriate use of anti D Ig, and the continued supply of a safe and efficacious product for prophylaxis.

Work-up of a Case of Early Onset IUGR

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Early Onset IUGR or IUGR setting in before 32 weeks gestation is less often seen than Late Onset IUGR. Previously a number of terms were used to denote Early Onset IUGR such as symmetrical IUGR, etc. of late term Early Onset IUGR is preferred. IUGR continues to remains a significant cause for perinatal mortality, NICU admissions and subsequent CNS disorders in childhood. Whereas Late Onset IUGR mostly follows conditions such as pregnancy induced hypertension, Early Onset IUGR can occur due to chromosomal anomalies, intrauterine infections and long standing maternal diseases such as chronic hypertension, collagen vascular disorders etc. Fetal congenital malformations can also cause Early Onset IUGR.

Work up of a case of Early Onset IUGR includes investigations aiming to find out possible etiology. Besides studies for maternal infection, infections capable of transplacental transmission including TORCH group are carried out. In order to exclude fetal congenital malformation a repeat organ targeted ultrasonography is warranted. Doppler flow studies in major fetal vessels often turn out to be normal inspite of presence of IUGR. Detail ultrasound to look for soft tissue markers for fetal aneuploidy is helpful.

However, obtaining cord blood sample by percutaneous transabdominal fetal blood sampling for chromosomal culture is indicated to exclude chromosomal disorders. Blood sample obtained by cordocentesis can also be used to look for TORCH infections, acid-base status of the fetus

Early Onset IUGR includes investigations aiming to find out possible etiology. Besides studies for maternal infection, infections capable of transplacental transmission including TORCH group are carried out. In order to exclude fetal congenital malformation a repeat organ targeted ultrasonography is warranted. Doppler flow studies in major fetal vessels often turn out to be normal inspite of presence of IUGR. Detail ultrasound to look for soft tissue markers for fetal aneuploidy is helpful.

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IUGR –anemia, Multiple Micronutrient Supplementation and Beyond

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Implication and Management of Oligohydroamnios and Polyhydroamnios

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Placental Pathology-Fetal Implications

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It is to be emphasized at the outset, that all placental lesions must be assessed in terms of their functional significance and that no abnormality of the placenta, however pathologically impressive it may appear, is of any importance unless it can be shown directly to impair the continuing growth and development of the fetus.

The placenta has a considerable functional reserve capacity, easily repairs ischaemic damage, is able to compensate for toxic injury and does not appear to age. Most of the macroscopically visible abnormalities of the placenta are of no functional significance, the major exception to this general banality being the uncommon large haemangioma which can cause complications in the mother, fetus and neonate. Most of the historical abnormalities seen in the placental villi represent a reaction to alterations in either maternal or fetal blood flow through the placenta, but a failure of adequate maturation of the villous tree may impair the functional efficiency of the placenta, as may defective trophoblastic differentiation. Infections of the placenta are important but do not influence placental function, while there is currently no firm evidence that the placenta ever suffers immune-mediated damage.

Intrinsic placental 'insuffiency' is extremely rare and it is becoming increasingly clear that this clinical syndrome is usually due to a restricted supply of maternal oxygen and nutrients as a result of inadequate transformation of the spiral arteries into uteroplacental vessels. This failure of placentation represents an abnormality of the relationship between fetal and maternal tissues at a relatively early stage of pregnancy, and it is only by gaining a better understanding of this relationship that the problems posed by such conditions as pre-eclampsia and idiopathic intrauterine growth retardation will be addressed in the presentation.

Management of B -Thalassemia in Pregnancy: Obstetricians Alert

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ß-thalassaemia is one of the world's most wide spread monogenetic disorders affecting over 30 million people worldwide. Advances in the management of ß-thalassaemia major by optimal blood transfusions and chelation therapy have improved survival of patients into adult life. Due to the prolonged life expectancy and improvements in quality of life, pregnancy has now become an important issue for patients and clinicians,

We report our experience of 11 women with ß -thalassaemia major over the last 15 years at the University College Hospital, London. The major pre-pregnancy issues including pre-pregnancy counseling, partner screening, medications, suitability for induction of ovulation and pregnancy care are reviewed.

Pregnancy was advised when patients had echocardiographically normal resting left ventricular performance. Iron overload was assessed by cardiac and hepatic MRI. All conceptions occurred after ART, as all had hypogonadotrophic hypogonadism, consequent on transfusional haemosiderosis, which is a common problem in ß-thalassaemia patients. Cardiac function, along with hematological, endocrine, and hepatic parameters were initially assessed and monitored throughout pregnancy and for 2 to 9 years post partum. All patients were screened for Hep B, Hep C and HIV prior to ART. Partners were investigated for haemoglobinopathy. Desferrioxamine or oral chelators were discontinued during pregnancy.

Fourteen healthy newborn infants were delivered. There were 2 sets of twin and one set of triplet pregnancy. One patient developed thromo-embolic episode, while 2 had pre-eclamsia. The mean serum ferritin concentration increased from pre=pregnancy value of 2000 to 5000 µg/L post delivery. Although no significant cardiac complications were encountered, the incidence of preterm labour and growth restriction were 3 fold higher than the background population. Elective caesarean section was performed in 73% of cases. None of the fetuses had congenital malformations. The mean fetal birth weight was 2.5 kg. Breast feeding was encouraged in all cases. Chelation therapy was recommenced in the immediate post partum period.

In conclusion, pregnancy is feasible in women with ß -thalassemia with normal resting cardiac performance and optimized iron overload status. Our experience suggests that, with proper care and guidance, pregnancies in women with thalassemia major are practical and can have successful outcome in specialist centres under a multi disciplinary team.

Feto Maternal Workup of Non Immune Hydrops

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Non immune hydrops is invariably a lethal or severely morbid condition & may result from anatomical or physiological alterations in a fetus. The exact incidence in our subcontinent is yet to be deciphered. A preliminary data got from the Birth Defects Registry of India would be presented. A few survivors have been reported & they would have had a self limiting etiology like infection.

Definition: Non immune hydrops is defined as a state where there is extracellular fluid accumulation in 2 or more body cavities in the fetus & there are no redcell antibodies circulating in the mother & fetus

Causes :

I trimester

1. Chromosomal anomaly – Most commonly Aneuploidy -Turners syndrome
2. Skeletal dysplasia – eg Achondrogenesis
3. Severe lower urinary tract obstruction – Prune belly syndrome

II trimester – Most common time for pick up

1. Structural cardiac defect in the fetus – Complex cardiac defects, Ebsteins anomaly, cardiomyopathy etc.,
2. Defects in the respiratory tract – Primary pleural effusion, high airway obstruction, congenital cystic adenomatoid malformation of the lung etc.,
3. Skeletal Dysplasias, Neromuscular syndromes etc.,
4. Metabolic causes – Lysosomal storage disorders, Red cell enzyme defects
5. Vascular – Twin to twin transfusion ,Trap sequence, Placental Chorioangioma etc.,
6. Infections – TORCH, Parvovirus, Syphilis etc.
7. Endocrine disorders – Fetal goitre

III trimester:

1. Later evolving cardiac, respiratory problems
2. Arthrogryposis, Pterygium syndromes
3.Rhythm abnormalities
4. Cloacal anomaly etc.,

List of maternal investigations in NIH

1. Blood group,Indirect Coombs test
2. TORCH titres- IgM /IgG
3. Kleihaur Betke test
4. Parental Haematology & Electrophoresis
5. Maternal Liver function tests
6. Parental karyotype – If fetus has a structural rearrangement

List of Fetal investigations

1. Haematogy, electrophoresis, direct Coombs test
2. Infection screen, Viral culture
3. Karyotype
4. Lysosomal enzyme screen
5. Liver function test
6. Biochemical analysis of pleural/ Ascitic fluid
7. Autopsy

Postnatal followup

1. Surgical correction options for structural defects
2. Continue therapy for functional defects
3. Periodic developmental assessment

PrePregnancy Counseling

Is a must in all couples with a previous hydropic child, family history of genetic disorders. This goes a long way in early detection & intervention in subsequent pregnancies.

Recognition and Management of Thyroid Disease in Fetus

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Diabetes Mellitus: Prepregnancy and Monitoring for Prevention of Congenital Malformations

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Diagnosing type 2 diabetes mellitus is very difficult during pregnancy because severe forms of GDM have similar clinical characteristics. On the other hand, it is not unusual for women tentatively diagnosed with GDM in early pregnancy to be found to have overt diabetes after delivery. Although a first-

trimester HbA1C value of 8% is highly suggestive of preexisting type 2 diabetes, definitive diagnosis of type 2 diabetes must be made after pregnancy using the 75-g, 2-hour glucose tolerance test.
Congenital malformations remain the major cause of morbidity and mortality among the offspring of women with diabetes. The incidence of multiple congenital malformations amid infants of mothers with insulin dependent diabetes mellitus (type I or IDDM) is between 6% and 12%. This is two to five times the incidence in non-diabetic mothers. The most frequently seen types of malformations in infants of diabetic mothers involve the cardiovascular, skeletal, central nervous, gastrointestinal and genitourinary systems, with cardiac malformations being the most common.
Both human and animal studies have demonstrated that diabetic embryopathy is associated with hyperglycemia during the period of organogenesis. However, the precise mechanism(s) by which hyperglycemia induces diabetic embryopathy remains unclear. A number of hypotheses have been proposed, such as alterations of fuels or fuel-related factors, arachidonic acid and myo-inositol deficiency, and an excess production of free oxygen radicals relative to the antioxidant reserve (ie, oxidative stress).
Because birth defects occur during the critical time of organogenesis ie 3-6 weeks after conception, preconception counseling and metabolic control before pregnancy reduces the rate of congenital malformations and the burden of suffering in offspring.
HbA1c level of less than 7% is a central feature of the pre-pregnancy monitoring. It is necessary that all the factors, which influence this level, be targeted, but particular emphasis is placed on diet and exercise and very regular contact with the health care team. Ideally any woman with diabetes undergoing care for infertility should consult with her diabetologist and have optimal glycemic control prior to commencing treatment. Most human studies have used first-trimester glycosylated hemoglobin levels as an indicator of glycemic control during organogenesis. The incidence of major structural anomalies has been shown to increase with increased levels of HbA 1c.
A systematic approach to pregnancy planning, avoiding unintended pregnancies through contraception, repeated emphasis by providers on the importance of pre-conception care & use of folic acid are essential components of a comprehensive diabetic management programme.
The development of very short acting Humalog insulin has enabled these women to decrease post-prandial blood sugar levels very quickly to normal. With the team of diabetes educators, dietitians, perinatologists, obstetricians, and endocrinologists, the patient will have the most up-to-date and thorough care as well as instruction in how to control her diabetes during the different stages of pregnancy. Having a patient with diabetes who wants to become pregnant involves planning by the patient and her team of professionals in order to achieve a healthy outcome.

Fetal Echo in the First Trimester and Early Second Trimester

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MRI in Prenatal Diagnosis Beyond 4D Ultrasound

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Chromosomal Anomalies First and Second Trimester Ultrasound

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Chromosomal abnormalities are a major cause of perinatal death and childhood disabilities. Consequently the diagnosis of chromosomal disorders constitutes most important indication of invasive prenatal diagnosis. However these invasive tests of amniocentesis, chorionic villous sampling or cordocentesis is associated with a risk of miscarriage of 1%. Therefore these tests are carried out in pregnancies at high risk for chromosomal defects.

Screening using ultrasound is based on the fact that most fetuses with chromosomal abnormalities have major structural malformations or minor abnormalities that can be detected on ultrasound.

Noninvasive Screening methods for chromosomal abnormalities

Maternal age 35 or above

Genetic history (previous trisomic offspring, other genetic diseases, parental translocation carrier)

Ultrasound at 11 – 14 weeks: Nuchal translucency screening

Ultrasound at 18 – 20 weeks: the genetic sonogram

Maternal serum biochemistry in 1st trimester (11 –14wks)

Maternal serum biochemistry in 2nd trimester (15 –20wks)

Fetal cells in maternal circulation (Investigational)

Risk of Trisomies

Maternal age: Older the mother the higher the risk of trisomies

The earlier the gestation the higher the risk of trisomies

Women with previous fetus or baby with trisomy 21, the risk in current pregnancy is 0.75% higher than her background risk

11 – 14 week Scan

Fetal Nuchal Translucent thickness

Nasal bone

Growth restriction - crown to rump length

Fetal Heart rate

Ductus Venosus flow

Fetal Doppler studies

Nuchal Thickening/Translucency

Nuchal translucency is the thickening of the nuchal soft tissues – subcutaneous fluid collection in the back of neck. This is measured in 1st trimester between 11 – 13 weeks (optimal time 13 weeks); in sagital scans and is correlated to crown rump length. The measurement involves the width of lucency alone, excluding the width of surface or occiput. The cut off is 3mm.

Nicolaides and coworkers have been instrumental in demonstrating their association with chromosomal anomalies - Trisomy 21 (most common), Triploidy, Trisomy 13, 18, 22, Tetrasomy 12 p.

Nuchal translucency in Trisomy 21:

NT measurements when combined with maternal age identifies up to 80% chromosomal anomalies with 5% false positive. When combined with free beta hcg and pregnancy associated plasma protein A (PAPP-A) sensitivity increases to 85% with false positive rate of 5%.

Most fetuses that have thickened nuchal folds in 1st or 2nd trimester may show resolution over period of time. Progression from an abnormal nuchal thickening to normal one is not necessarily indicative of a normal karotype. Fetuses with nuchal abnormalities need amniocentesis even if this resolves.

Fetal nuchal translucency and other chromosomal abnormalities:

Trisomy 18:

In addition to increased NT there is early onset intra uterine growth restriction (IUGR), bradycardia; and in 30% associated omphalocele. The biochemical markers of maternal serum free beta hcg & PAPP-A decreased.

Trisomy 13:

In addition to increased NT there is fetal tachycardia in about 2/3rd of cases, early onset intra uterine growth restriction (IUGR), holoprosencephaly or omphalocele in about 30% cases. The biochemical markers of maternal serum free beta hcg & PAPP-A decreased.

Triploidy:

Increased NT is seen along with early onset asymmetric intra uterine growth restriction (IUGR),

relative bradycardia, holoprosencephaly, omphalocele, posterior fossa cyst in 40% of cases. Molar changes in placenta are seen in about 33% cases.

Turner's Syndrome:

Along with increased NT there is fetal tachycardia in 50% of cases, and early onset intra uterine growth restriction.

Absent/hypoplasia of Nasal Bone:

is seen in 65 –70% of Trisomy 21, 50% in Trisomy 18 and 30% in Trisomy 13.

In screening for trisomy 21 at 11 – 14 weeks: The combination of sonographic markers of nasal bone & nuchal translucency with biochemical markers of free beta hcg, PAPP-A could result in a detection rate of about 97% for a false positive rate of 5%, or detection rate of 95% with false positive rate of 2%. Chromsomally normal fetuses showed a nasal bone in 99.5% of cases

Growth Restriction (First trimester):

Crown rump length

Low birth weight is a common feature of many chromosomal malformations. Prenatal studies in 2nd & 3rd trimester have reported high prevalence of aneuploidies in severe IUGR.

In the 1st trimester trisomy 18 & triploidy are associated with moderate to severe growth restriction; trisomy 13 & Turner's syndrome with mild growth restriction. There is essentially normal growth pattern in Trisomy 21.

Study by Bahado & Singh who showed the risk of aneuploidy increases when CRL is shortened by 14mm from that expected for the period of amenorrhea.

Fetal Heart Rate

Normal Pregnancy

5 weeks	110 per minute
9 weeks	170 per minute
14 weeks	150 per minute
Trisomy 13	Tachycardia
Turner's syndrome	Tachycardia
Trisomy 18	Bradycardia
Triploidy	Bradycardia
Trisomy 21	Mild increase in rate

Ductus Venosus

Absent to reversed a wave is seen in 60 – 90% of chromosomally abnormal fetuses

Only 5% of chromosomally normal fetuses showed abnormal a wave.

Fetal Doppler Studies

Umbilical artery

Two studies reported no significant difference in umbilical artery flow between trisomy 21 & normal fetuses.

One study reported about 50% of trisomy 21 fetuses between 11 – 14 weeks showed abnormal umbilical artery flow with Pulsatility index above the 95 percentile.

Umbilical vein

The Doppler study in 11 – 14 weeks reported pulsatile flow in 25% of normal fetuses and in 90% of fetuses with trisomy 18 & 13. In trisomy 21 the pulsatile venous flow was not significantly different from normal.

Structural anomalies in 2nd trimester with higher probability of chromosomal malformations

Central Nervous System:

Holoprosencephaly:

Holoprosencephaly is a spectrum of severe early abnormalities in forebrain cleavage.

40 – 60% of these are associated with chromosomal malformation. Trisomy 13 or Variant of Trisomy 13 account for 50% of all cases. Also seen in Triploidy.

Prenatal sonographic diagnosis is based on demonstrating a single dilated mid line ventricle.

Holoprosencephaly is commonly associated with mid facial abnormalities. The risk of chromosomal defect is higher when this is associated with extra facial anomalies.

Dandy Walker Complex:

Is a spectrum of abnormalities of cerebellar vermis, cystic dilatation of 4th ventricle and enlargement of cisterna magna.
Dandy Walker Malformation: is complete or partial agenesis of cerebellar vermis and enlarged posterior fossa.
Dandy Walker Variant: Partial agenesis of cerebellar vermis without enlargement of posterior fossa
Mega cisterna magna: Normal vermis and 4th ventricle.
The overall incidence of chromosomal malformation is about 40% usually Trisomy 18 & Trisomy 13.
Study by Chang et al: reported 52% chromosomal defects with DWV and 32% with DWM.

Ventriculomegaly:
The overall incidence of chromosomal abnormalities is about 10%. Commonly associated with Trisomy 18, 13, 21; Triploidy; Translocation.
The incidence is higher with mild to moderate ventriculomegaly.

Agenesis of Corpus Callosum:
Is difficult to detect before 22 weeks and is suspected by the absence of cavum septum pellucidum and enlargement of posterior horn of lateral ventricle (tear drop sign).
Chromosomal risk in Prenatally diagnosed cases is about 10%; and in postnatally diagnosed cases 4%. The associated Chromosomal abnormalities are trisomy 8, 11, 13, 14, 15, 18; triploidy and translocation.

Microcephaly:
Prenatal diagnosis is based on identification of disproportionately reduced head circumference. The chromosomal defect usually trisomy 13 is seen in about 15% of cases. Usually associated with other defects.

Choroid plexus cysts:
relatively a common variant with no effect in development. These are detected between 16 - 24weeks of gestation and generally resolve by 28 weeks of gestation.
These have been found commonly in association with trisomy 18.
Factors in calculating the risk of chromosomal Malformation in isolated choroid plexus cysts is maternal age; size of cyst (more than 10mm) and delayed resolution of these cysts.

Brachycephaly:
is seen in trisomy 21 and is relative shortening of the occipito frontal diameter. It is a non specific marker and generally associated with other sonographic markers; may be seen in normal fetuses as well.

Strawberrry shaped skull:
Strawberrry shaped skull is seen in 80% of fetuses with trisomy 18. The strawberry shaped head shows flattening of the occiput and narrowing of the frontal part of head.
A Non specific marker and generally associated with other sonographic markers.

Facial Abnormality:
Cleft Lip and Palate is associated commonly with trisomy 18 & 13.
Postnatally chromosomal abnormalities are seen in less than 1% of babies with facial clefts. In the prenatal series the incidence is about 40% and is commonly associated with Trisomy 18 & 13.
Hypoplasia of nasal bone is a sensitive and specific 2nd trimester marker for trisomy 21. This is defined by a nasal bone that is not visible or the length is less than 2.5mm.
This is seen in 65 – 70% of cases of Trisomy 21; 50% Trisomy 18 & 30% of Trisomy 13.

Ear abnormalities:
In about 85% of neonates with trisomy 21 the ear length is below the third percentile of the normal range. Small and deformed ears are seen in Trisomy 18, 13 & 21.
Micrognathia: is a non specific finidng and is seen in trisomy 18 & triploidy. The incidence of chromosomal abnormalities is about 60%. All fetuses have other malformations and/or growth retardation.
Macroglossia: Postnatally the macroglossia is a common feature of trisomy 21. Antenatally is rarely diagnosed unless the other features of trisomy 21 are present.
Neck
Cystic hygroma: Prenatal sonographic diagnosis is based on demonstration of bilateral septated

cystic masses in the occipiocervical region. Chromosomal defect is seen in 75% of cases and is almost always Turner's syndrome.

Nuchal edema or nuchal fold is the 2nd trimester equivalent of nuchal translucency at 11 – 14 weeks. This is measured in transverse scans in 2nd trimester with the cut off taken as 5mm up to 20 weeks. Increased nuchal fold thickness is found in 33% of fetuses with trisomy 21 and in 0.6% of chromosomally normal fetuses.

Chest:

Diaphragmatic hernia may be sporadic but in 50% this may be associated with chromosomal abnormalities. Prenatal diagnosis is based on the demonstration of stomach, intestine or liver in the thorax and the associated mediastinal shift to the opposite side. The incidence of chromosomal defects mainly trisomy 18 is about 20%.

Cardiac abnormalities:

Heart defects are seen in more than 90% of fetuses with trisomy 18 & 13; 50% with trisomy 21; 40% with Turner's syndrome. The associated cardiac defects are atrio ventricular septal defect (endocardial cushion defect – most commom), Double outlet right ventricle, Tetrology of Fallot, Hypoplastic heart, VSD, Aortic coarctation.

Intra cardiac echogenic foci (EIF): are seen in the four chamber view of heart. 90% of these resolve by third trimester. These are seen in 25% of fetuses with trisomy 21 and in 4% of normal fetuses. Risk of Aneuploidy increases with multiple foci, large EIF, in both right & left ventricle, in right ventricle alone. There is two fold increased risk with EIF in
RV & both ventricles.

Abdomen:

Omphalocele: Prenatal diagnosis is based on the demonstration of mid line anterior abdominal defects, the herniated sac with the umbilical cord insertion at the apex of the herniated sac. The risk of chromosomal defect is 4 times higher when the herniated content is bowel.

The associated chromosomal defects mainly trisomy 18 & 13 are seen in 60% of cases at 12 weeks; 35% of cases at mid gestation and 15% in neonates.

Esophageal Atresia:

Prenatally chromosomal defect mainly trisomy 18 is seen in 20% of cases. This is suspected when there is polyhdroamnios, non visualization of stomach in repeated sonographic examinations.

Postnatally the chromosomal defects are reported in 3 – 4% of neonates with esophageal atresia.

Duodenal atresia:

Diagnosed on ultrasound by the characteristic "double bubble" appearance of dilated stomach and proximal duodenum and commonly associated hydroamnios. This is associated with Trisomy 21 in 40% of cases.

These changes are seen after 25 weeks.

Echogenic bowel:

may be seen in trisomy 21, 18, 13 and triploidy. The incidence of echogenic bowel in trisomy 21 is about 13%.

Urinary tract abnormalities:

The risk of defect is similar whether unilateral or bilateral, different renal anomalies, uretheral or ureteral obstruction and oligoamnios. Incidence of chromosomal defects in females is twice that in males.

Mild hydronephrosis is commonly seen in Trisomy 21;

Moderate/ severe hydronephrosis, multicystic kidneys, renal agenesis is seen in trisomy 18 & 13.

Skeletal abnormalities:

Syndactyly 3rd & 4th digit: Triploidy

Polydactyly: Trisomy 13

Clinodactyly & sandle gap: Trisomy 21(hypoplasia of middle phalynx of 5th digit)

Overlapping fingers, rocker bottom feet, talipes: trisomy 18.

Short humerus & femur are associated with Trisomy 21, 18, triploidy and Turners's syndrome

Fetal growth restriction: Low birth weight is seen in many chromosomal defects, but the incidence of small for gestation age neonates is about 1%.

IUGR is commonly seen in triploidy and trisomy 18.

The highest incidence of chromosomal defects is seen in cases where along with IUGR there are associated fetal structural abnormalities, amniotic fluid volume is normal or increased, and in the group which shows normal waveforms for both uterine and umbilical arteries.
Detection rate, false positive rate for different screening combinations

	DR(%)	FPR (%)
MA + > 35years	33	10
MA+ AFP+ hcg+ uE3 (15 – 17 weeks)	60	5
MA+ NT (11 – 14weeks)	80	5
MA+ free beta hcg +PAPP-A	60	5
MA+ NT + free beta hcg +PAPP-A	85	5
MA+ NT + NB	92	3
MA+ NT + NB + free beta hcg +PAPP-A	97	5

(AFP: alpha feto protein; DR: detection rate: FPR: false positive rate; hcg: human chorionic gonadotrophin; MA: maternal age; NB: nasal bone; NT: nuchal translucency; PAPP-A: pregnancy associated plasma protein – A; u E3: unconjugated estriol)
(Source: Rumack).

Skeletal Dysplasia

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Non Invasive Prenatal Diagnosis of β- thalassemia and RH-D Already a Reality

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Fetal cells, specifically fetal erythroblasts, isolated from the maternal circulation offer the possibility of obtaining fetal genetic material in a risk-free non-invasive manner. Prompted by a number of high-profile publications, the NIH decided to fund a large scale study, the so-called NIFTY study, to investigate the feasibility of this approach. The data from this study, in which our laboratory participated, indicated that current technologies are not suitable for clinical applications, as only low levels of sensitivity could be attained. During these investigations we noticed that isolated fetal erythroblasts could be more efficiently analysed by single cell PCR than by FISH.
A major focus in our laboratory over the past years has been the analysis of cell-free fetal DNA in maternal plasma. Although the presence of cell free circulatory nucleic acids was first reported almost 60 years ago by Mandel and Métais, it is only in recent years that this phenomenon has gained the interest of the wider scientific community, especially in oncology and prenatal medicine. The analysis of cell-free fetal DNA in maternal plasma is particularly suited for the examination of fetal genetic loci completely absent form the maternal genome, such as the Y chromosome or the RhD gene in Rhd negative pregnant women. Indeed, this approach has been found to be so reliable that it is already being used clinically in several European centres for the detection of the fetal RhD status in pregnancies with a Rhesus constellation and sex in pregnancies at risk for an X-linked disorder.
The detection of other fetal genetic loci which differ only slightly from maternal ones has proven to be more problematic. In this regard we have recently made the observation that circulatory fetal DNA sequences are generally smaller than comparable maternal ones, and that by selecting sequences with a small size (<300 bp) a selective enrichment of fetal DNA sequences can be attained. By using this approach we have recently shown that fetal point mutations, such as those for b- thalassemia, can be reliably determined from circulatory fetal DNA.

Prenatal Diagnosis of Hemoblobinopathies in India

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Hemoglobinapthies are disorders caused by the presence of abnormal hemoglobin production in the blood. b-Thalassemia is the commonest single gene disorder which is Autosomal recessive characterized by chronic hypochromic hemolytic anemia and by dependence on blood transfusion to sustain life. It is caused by mutations in the b-globin gene that reduce or abolish the synthesis of b-globin chains required for the formation of adult hemoglobin (Hb, A, a_2b_2).
In the eastern states b-thalassemia/Hb E is common and behaves as b-thalassemia major (while homozygous Hb E is fairly innocuous). Sickle cell disease is prevalent mostly in tribal communities in central India, Maharashtra, Gujarat, Rajasthan, Nilgiri hills and Wayanad district of Kerala. Sickle cell / b-thalassemia combination occurs to a small extent, and has moderately severe manifestations. Among Punjabis Hb D is observed in a 2-3% of people from Punjab, but does not lead to significant manifestations when combined with b-thalassemia gene.
Treatment of thalassemia is expensive, arduous and painful. It is associated with complications of chelation therapy, and the risk of transfusion-related infections. It is, therefore, necessary to prevent the birth of affected children by prenatal diagnosis, to reduce the socio-economic pressure on the family and burden of the disease on the community. Therefore, genetic counseling and prenatal diagnosis (PND) are essential to control this dreaded diseases.
The various elements for a successful prenatal diagnosis program for hemoglobinopathies in India will be discussed which include :

- Establishing accurately that both the partners are carries
- Molecular studies in the affected child and the parents to identify the mutations in the b-globin gene that cause the disease.
- Choosing the most suitable obstetric technique to obtain fetal tissue and performing the procedure.
- Extraction of DNA from the fetal tissue and carrying out molecular studies to establish the status of the fetus.
- Sources of error and their avoidance.
- Verification of the result after birth or abortion.

Molecular Fetal Diagnosis of Neuromuscular Disorders

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Neuromuscular disorders are a group of conditions affecting either muscles, nerves which pass from brain and spinal cord to the muscles, or complex junction between muscle and nerve. Most neuromuscular disorders result from genetic abnormality and cause progressive weakness. Some are severe and life expectancy is limited to first or second decade whereas others are relatively mild. For severe conditions like Duchenne muscular dystrophy and spinal muscular atrophy, prenatal diagnosis and carrier testing are the only option for the family.
Duchene muscular dystrophy and its milder form Becker muscular dystrophy result from mutations in dystrophin gene located on Xp21 region. Deletion mutations in the two hotspot regions of the gene account for genetic abnormality in approximately 65% patients and prenatal test can be done with certainty. However, if no deletion is detected, prenatal diagnosis involves DNA based linkage analysis generally administered in familial cases. Carrier detection in female relatives of index patient can also be carried out by linkage analysis. These tests are routinely carried out at several centers in India.
Spinal muscular atrophy is second most lethal disorder after cystic fibrosis. It is autosomal recessive and involves degeneration of anterior horn cells of spinal cord. Survival motor neuron gene *(SMN)*

has been identified to cause SMA. *SMN* gene is present in two homologous copies: telomeric (*SMN1)* and centromeric *(SMN2*). A PCR RFLP based DNA test is used for disease diagnosis and prenatal testing. However, quantitative PCR analysis is required for carrier testing.
In recent years, genes for various other neuromuscular disorders have been identified and genetic testing is available in some of them. Apart from DNA testing, immunocytochemical approaches can be used for definitive diagnosis because mutation spectrum in most instances is quite heterogeneous. At present, there is, as yet, no way of greatly affecting the long-term course of any of these diseases but molecular fetal diagnosis, if available, is helpful in reducing disease burden.

Pre-natal Diagnosis and Carrier Detection in Haemophilia A

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Haemophilia A is characterized by deficiency of procoagulant factor VIII (FVIII). Affected males suffer from joint and muscle bleeds and easy bruising, the severity of which is closely correlated with coagulation factor VIII activity (FVIII:C) in their plasma. Patients with <1%, 1-5% and 5 to 30% of coagulant factor VIII activity are considered to have severe, moderate and mild disease respectively. Since hemophilia A is an X linked inherited disorder, the risk to other family member of the proband depends on the carrier status of the female relatives. Therefore carrier detection and genetic counseling play an important and integrated part of the comprehensive care for hemophilia A. A pedigree has to be drawn up to show the relationship of a potential carrier to any family member with hemophilia A. Based on pedigree, potential carrier can be classified as obligate carrier and probable carries. Families with only one family members is known to have hemophilia are sporadic or isolated cases and they constitute a special problem. Carriership can also be determined based on the FVIII:C and vWF:Ag levels. Individuals with values less than 0.7 for FVIII: C and vWF;Ag ratio are considered as carriers. Linkage analysis is the method most commonly used to determine female carrier status in families with haemophilia A. It is an indirect method of tracking the chromosome (allele) that carries the mutant gene, this can be achieved by Restriction Fragment Length Polymorphisms (RFLPs) and Tandem repeats (VNTRs). In our study at the Department of Haematology, AIIMS, the limitation of linkage analysis have been partially overcome by using FVIII:C /vWF Ag estimation together with linkage analysis to determine the carrier status of female relatives of sporadic patients with Hemophilia A. The direct gene analysis involves detection of inversion mutation which is present is approximately 45% of sever hemophilia A. Prenatal diagnosis can be done either in chorionic villi or in cord blood by direct/indirect gene analysis. The chorionic villi sampling (CVS) is performed at 10 to 16 weeks' of gestational age. Its advantage is that it gives an early diagnosis. The indirect gene analysis of CVS is done using informative linkage markers (Bcl1, Xba1, CA13 and CA22) where there is positive family history. The direct gene analysis of CVS is done for intron 1 and 22 inversion mutations in case of sporadic cases. After the CVS, before proceeding for molecular analysis, maternal contamination in the CV need to be excluded by either VNTR PCR method or APO B method. The results of the above will be discussed. In conclusion, it is possible to detect carriers of haemophilia and to diagnose haemophilia A by molecular techniques. There is a need to increase the awareness of availability of these techniques in order to control the prevalence of Haemophilia A.

Prevalence of Genetic Counseling in Male Infertility

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Role of Art in Fetal Medicine

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Genetic Disorder Are Not So Uncommon, Usually Affects 1-3 % Of Individuals At Birth. There Are Other Conditions Like Rah Incompatibility Etc. Which Affects A Growing Fetus? Some Of The Disorders Are Not Compatible With Life, Others Produce Life Time Morbidity. There Are Two Approaches to tackle The Problem. One Is Prevention Of Prevention Of Genetic Disorder, Other Is Treating The Condition.
Assisted Reproductive Techniques Like In-Vitro Fertilization / ICSI / Donor Insemination Have Been Used For Prevention Of Some Of The Genetic Disorders & Provides One Of The Best Alternative To Avoid Genetic Disorders. Use Of Donor Gametes Of One Or Both Partners In Cases Of X Linked Disorders Can Successfully Prevent Genetic Transmission .Of X Linked Disorders To Offsprings . Males Having Cystic Fibrosis Mutation Syndrome May Be Advised To Use Donor Semen To Avoid Transmission. Same Way If Both Partners Are Having Thalesimia Trait, Chances of Having a Thalesimia Major Offspring Can Be Avoided By Simply Using Donor Sperms. Other Non X Linked Single Gene Defects Like Sickle Cell Anemia , Tay Sach,S Disease & Hemophilia Can Also Be Avoided Using Art Procedures .
In Cases Of High Risk Rh Incompatibility, Use Of Rh -Ve Donor Sperms Can Be Used To Avoid Further Perinatal Mortality.
PGD Has Revolutionized The Role Of Art To Avoid Genetic Defects. Now It Is Possible To Screen Embryos Before Implantation For Many X-Linked Disorders / Defects In Numbers / Translocation & Single Gene Defects By Using FISH /PCR On Embryo Biopsy & Only Defect Free Embryo Be Transferred To Uterus . Many More Gene Specific Probes Are Going To Be Available In Near Future Which Can Be Used For PGD, Helping To Avoid The Occurrence Of Any Genetic Disorders.

PND Issues in IVF, ICSI, Surrogacy

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Preimplantation Genetic Diagnosis – Indian Experience

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Preimplantation Genetic Diagnosis (PGD) is a very early form of prenatal diagnosis. It is a procedure during an IVF-ICSI cycle, where one blastomere is biopsied from a 6-8 celled embryo and tested for genetic abnormalities such as aneuploidies or translocations by fluorescence *in situ* hybridization (FISH). Only the normal embryos are transferred to the mother. This technique is in its infancy in India, though a few thousand babies have been born worldwide. PGD for molecular genetic disorders such as Thalassemia by single-cell PCR is not yet done in India.
We have the expertise and facilities to carry out PGD for 5-9 aneuploidies and inherited translocations, by FISH. Out of 18 IVF cycles for PGD, we have biopsied 72 blastomeres, and transferred 29 normal embryos, resulting in 2 clinical pregnancies. Fixation of a single cell on the slide is a crucial step, since an intact nucleus devoid of cytoplasm gives the best results after FISH. This technique has to be perfected on arrested embryos, before attempting PGD. The trauma of aborting an abnormal fetus detected at conventional prenatal diagnosis is avoided by PGD.

Advances in Cytogenetics Applied to Prenatal Diagnosis

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QF-PCR As A Stand Alone Test

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Karyotype analysis of cultured fetal cells has been regarded as the gold standard for cytogenetic prenatal diagnosis for 35 years. This method has proved to be highly reliable for identifying chromosome copy number abnormalities (aneuploidy) and large structural rearrangements in fetal cells obtained invasively by amniocentesis or chorionic villi sampling (CVS). Screening tests for fetal aneuploidy are now widely used and include biochemical maternal serum tests and ultrasonography on large populations of pregnant women. As a result, there has been an increased demand by all parties for more rapid diagnostic methods that do not require cell culture. Fluorescence in situ hybridisation (FISH) on interphase cells and quantitative fluorescence PCR (QF-PCR) have been introduced to allow rapid diagnosis (1 or 2 days) of the common aneuploidies (trisomy 13, 18 and 21 and aneuploidy of the sex chromosomes). Both methods have been shown to be robust in prenatal diagnosis; however, QF-PCR has the additional advantage of being much cheaper and allowing the processing of much larger number of samples than FISH. It has therefore replaced interphase FISH in an increasing number of genetic laboratories.
We retrospectively analysed the karyotypic results of 16.400 consecutive amniocenteses or CVS performed during 1999-June 2004 at the Karolinska University Hospital, Stockholm, Sweden. The frequency of expected undetected unbalanced karyotypes was determined according to indications for sampling, if quantitative fluorescence PCR (QF-PCR) had been used that includes microsatellite probes for chromosomes 13, 18, 21 and the sex chromosomes. Based on the results and validation studies of QF-PCR, a new policy of QF-PCR as a stand alone analysis was introduced and offered as an option to karyotyping in January 2005 when prenatal diagnosis is performed for advanced maternal age or anxiety. About 2600 prenatal QF-PCR analyses and only 600 karyotype were performed during 2005. The advantages and limitations of this approach will be discussed.

Rapid and Accurate Screening of Chromosomal Aneuploidies in Prenatal Diagnosis

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Chromosomal aneuploidies make a major contribution to human morbidity and mortality. Classical prenatal detection of chromosomal aneuploidies by conventional cytogenetics is time taking and labor-intensive. The present study aimed at increasing sensitivity and reducing the time between sampling and diagnosis by using Fluorescence In Situ Hybridization (FISH) analysis on uncultured cells obtained from amniotic fluid and chorionic villi sampling (based on gestational age at presentation). The pregnant women were classified as high-risk pregnancies on the basis of the following indications:- a previous child with chromosomal abnormalities, advanced maternal age (age >34 years),a fetal

abnormality detected through ultrasound examination, abnormal levels of maternal serum alpha-fetoprotein (MS-AFP) and b human chorionic gonadotropin (bhCG) or occurrence of a chromosomal abnormality in either of the parents. The twin pregnancy included for prenatal diagnosis had a hydatiform mole. The sample used for cytogenetic and FISH analysis was amniotic fluid and chorionic villi sample. The analysis of aneuploidies was also conducted in abortuses obtained from spontaneous abortion cases.

FISH analysis was done using commercially available probes for chromosomes 13, 15,16, 18, 21, 22, X and Y on uncultured cells. Simultaneously, conventional cytogenetics was done by long-term culturing of these tissues to analyze anomalies in chromosomes other than the ones for which probes were used. The results of the study demonstrate high sensitivity and specificity of the assay implying the potential use of these tissues for rapid and accurate prenatal and postnatal diagnosis. The hybridization effi.ciency of the 5 probes that were used for detection of aneuploidies in prenatal tissues was 100%. FISH analysis was successful in all the cases while conventional cytogenetic analysis could not be done in few cases. The results of interphase FISH were in conformity with cytogenetic results in all cases where cytogenetics analysis could be done. Hence, FISH analysis for screening most common aneuploidies in prenatal tissues could be a 'stand-alone test' for the same. However, this targeted approach should be used as an adjunct to conventional cytogenetics that remains a 'gold' standard for whole genome screening. Another highly sensitive analysis namely, Amnio-PCR has been recently introduced for analyzing chromosomal aneuploidies. The main advantage of this technique apart from high sensitivity, resolution and timesaving is that very small amount of prenatal tissue is required for analysis. Further many samples can be analysed at the same time. This technique also reduces the time between sampling and diagnosis.

To conclude, prenatal and postnatal diagnosis using interphase FISH and Amnio-PCR is a simple, efficient, reliable, reproducible and highly sensitive tool that can add to the diagnostic utility of conventional cytogenetics by decreasing the time interval between sampling and diagnosis and providing results within 24 hours. This is of tremendous value especially in urgent high-risk pregnancies and for proper management and genetic counseling.

Trouble Shooting in Prenatal Diagnosis of Chromosome Disorders

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The most common numerical abnormalities found in live births are aneuploidies of chromosome nos. 13,18, 21, X & Y. During Prenatal Diagnosis, these can be ruled out by Conventional Karyotyping or by Fluoresence Insitu Hybridisation of fetal cells (amniocytes / chorionic villi / cord blood).

Generally people who chose to have prenatal diagnosis (PND) are concerned about some chromosome condition, the most common of which is Down Syndrome due to high maternal age, or triple screening test being positive, or previous child being Down's,etc. . But, often there are different categories of unexpected chromosome results, such as:

1. A sex chromosome aneuploidy
2. Some autosomal trisomy other than +21
3. Polyploidy
4. Structural rearrangement
5. An extra structurally abnormal chromosome.
6. Mosaicism of any of the above categories.
7. Chromosome polymorphic variations.

Mosaicism may be rare, but poses a problem in prenatal cytogenetic diagnosis. Chromosome mosaicism detected in chorionic villus samples (CVS) or amniocyte culture does not necessarily truly reflect a constitutional mosaicism of the embryo. One needs to differentiate between constitutional, confined and pseudomosaicism. In any prenatal sample there can be level I, level II, Level III mosaicism. One

needs to decide which should be conveyed to the patient, during counselling.

Chromosome variations are also often seen and are often normal polymorphic variations. However, one odd case might be carrying a functional euchromatin at the region of chromosome variation.

Trouble shooting of such unexpected chromosome results will be discussed.

Minimally Invasive Autopsy

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Objectives: To determine the feasibility of a minimally-invasive autopsy based on post-mortem MRI and percutaneous organ biopsies as an alternative to full autopsy in stillbirth and fetal abnormality.
Methods: Study design: feasibility study
Participants: 30 fetuses from pregnancies that ended in miscarriage, stillbirth or termination for fetal abnormalities, with gestational ages of 16-39 weeks.
Interventions: Whole-body post-mortem fetal MRI, and percutaneous organ biopsy of major fetal organs, prior to conventional perinatal autopsy with explicit parental consent. Main outcome measures: interval from delivery to post-mortem MRI and autopsy; confidence scores of organ normality and abnormality on postmortem MRI as reported by consultant radiologists; proportion of cases in which organ biopsies where obtained from major fetal organs and in which they were adequate for histological assessment
Results: Median delivery-MRI interval was 1 day (range 0 - 4 days) and median delivery-autopsy interval was 4 days (range 2-11 days). Most fetal organs were given high scores for imaging confidence, except for cardiac and abdominal structures. Out of a possible 210 organ biopsies from seven target organs, 107 biopsies were obtained, of which 94 were adequate for pathological comment. Adequate samples from the fetal lung and liver were obtained in 86% (95% CI 68-96%) and 76% (95% CI 56%, 90%) of cases respectively.
Conclusions: Minimally-invasive perinatal autopsy is feasible. Most fetal anatomy can be evaluated with a high degree of confidence using postmortem MRI, with the possible exception of cardiac or gastrointestinal abnormalities. Percutaneous biopsies adequate for histological examination can be obtained from fetal organs such as liver and lungs in the majority of cases. Minimally-invasive autopsy does not require immediate access to specialist perinatal pathology services, and may reveal information not otherwise obtainable by conventional autopsy or by MRI alone.

Congenital Malformations: USG and Autopsy Correlation

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Fetal ultrasonographic examination has become an integral part of obstetric care. With easy availability and improved resolution of USG a number of malformations of various organ systems of the body are diagnosed prenatally. The ultrasonographic findings are reliable to take decision regarding continuation or termination of the current pregnancy and major discrepancy between USG findings and autopsy findings is rare. However, associated findings may be missed or may not be ultrasonographically detectable. Identification of these malformations is important to reach the final diagnosis of the malformation / malformation syndrome for counseling for the future.

It has been observed that in 20 to 30% cases fetal autopsy provides additional findings which are not detected on USG. This changes final diagnosis and risk of recurrence in half of the cases. The detection of associated malformations depends on ultrasonographer's knowledge of multiple malformation

syndromes. This helps in deciding what else to look for once a malformation is detected. Detailed examination findings of the previous child with multiple malformation syndrome helps greatly in correct USG diagnosis. Postmortem radiography is essential for accurate diagnosis of skeletal dysplasias. Similarly postmortem histopathological examination is essential for certain disorders like cystic renal disease and fetal infections.
Hence in spite of great improvement in fetal ultrasonography, fetal autopsy continues to remain essential for counseling for future pregnancies. Families for whom fetal autopsy is unacceptable, in addition to external examination and radiography, whole body MRI is an useful, but costly option.

Work-up and Management of a Congenitally Deaf Child

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Hearing loss is one of the most common birth defects affecting 3-4 per 1000 babies world wide.
Prelingual hearing loss, if unrecognised, has a major impact on a child and family, interfering language development and communication.
Congenital deafness can be inherited (genetic) or caused by environmental factors.
In around 60% cases, genetic causes are implicated, due to single gene mutations. Hearing loss may be **syndromic** when associated with defects in other organ systems. The majority of cases are isolated, i.e., **non-syndromic**. Non-syndromic deafness is extremely heterogenous with more than 100 genetic loci mapped and 50 genes identified till date. Autosomal recessive inheritance is found in 80 % cases.
Among all the genetic causes, mutation in a small gene, GJB2, contributes to 50-70% of ARNSHL in some ethnic populations, with predominance of a founder mutant allele, specific for a given population.
A genetic study for GJB2 mutations in a large cohort of Indian families segregating ARNSHL, revealed an overall prevalence of 20% , with a regional variation from 15 to 40%, and a founder mutation accounting for upto 80% mutant alleles of GJB2.
Linkage analysis may be carried out to identify involvement of other common deafness genes, in cases negative for GJB2.
Work-up of a congenitally deaf child includes a detailed antenatal and post-natal history, and TORCH studies to r/o environmental factors. Family history, pedigree charting and detail clinical evaluation are essential for clues to genetic etiology.
Genetic work-up include mutation analysis for GJB2 alleles, and if negative, linkage
studies for some common genes may be possible.
An accurate diagnosis is essential for proper genetic counselling, predicting risk of recurrence and prenatal diagnosis if requested.
Early diagnosis is crucial for implementation of rehabilitation and development of communication skills. With timely intervention and proper management it is possible to integrate a congenitally deaf child in normal school and offer education as good as a hearing child.

Newborn Screening for Inborn Errors of Metabolic Disorders

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Every Year, 4 million babies still die in their first four weeks of life, most from preventable causes. The number is double the deaths due to HIV/AIDS, although AIDS is rightly hailed as a global emergency, newborn deaths are largely ignored. While we neglect these challenges, 450 newborn children die every hour, mainly from preventable causes, which is unconscionable in the 21st century" – Lancet Neonatal Survival Series,3rd March,2005

A 6 day male infant suggestive of metabolic cardiomyopathy with history of similar disorder and death of male sibling at age 7 days, was admitted in sick condition. Child did not pass urine during 1 day of hospital stay. Dialysis was started after 12 hours of admission and the child succumed to death within 24 hours of resuscitation. We tested the blood by tandem mass spectrometry. The cause of death was Carnitine Palmitoyl Transferase Deficiency Type1- an inborn error of fatty acid oxidation.

We received blood sample of 10 day old male child, D.O.B 14 February,2005. This child developed tachypnea after first two feeds which resolved after 4 days. On refeeding again child had tachypnea and developed severe acidosis. Blood ammonia was raised . By testing with tandem mass spectrometry, cause was found to be Multiple CoA Carboxylase deficiency. As biotinidase deficiency can be one of reason, we tested serum for biotinidase activity. Child was found to have profound biotinidase deficiency. After start of biotinidase and other appropriate treatment, child became calm and accepted milk feed without any problem . Child is surviving fine now.

These cases are atypical in sense that the metabolic disorder was identified. Earlier due to lack of non availability of Tandem Mass Spectrometry in India, cause of neonates death due to metabolic disorder always remained unclear.

Tandem Mass Spectrometry is a effective technology that can be used to identify approximately 30 inherited metabolic disorders of aminoacids, ureacycle, organic acid and fatty acid oxidation from a single drop of blood. Tandem Mass Spectrometry is superior to other technologies as it allows mass screening of newborns for the presymptomatic detection of treatable metabolic disorders.

The incidence of metabolic disorder is on increase in India and is no longer a hypothesis. An estimated 9,760 infants are born with aminoacid disorders each year (Dr IC Verma, Community Genetics 2002;5:192-196). The need to screen newborns for metabolic disorders arises out of the fact that most cases take to irreversible brain damage as time progresses. Newborn screening in India has never been seriously viewed in India because of lack of awareness and paucity of facilities.

'That some infants aren't being caught and treated when possible is a national tragedy and Tandem Mass Spectrometry should be standard care for all newborns, much as blood pressure test is a part of every medical checkup", says Herry Hannon, Director of newborn screening services at the U.S. Centers for Disease Control and Prevention ("Early Warning. A Simple Test Saves One Baby;Another Falls Ill" The Wall Street Journal June17,2004).

Beside screening by Tandem mass Spectrometry for amnioacids,urea cycle disorders, organic acid, fatty acid disorders, we are also screening for Congenital hypothroidism, Galactosemia, Cystic Fibrosis, Congenital adrenal hyperplasia, Glucose-6-Phosphate dehydrogenase deficiency, Biotinidase deficiency.

Ignoring newborn screening can be painful tragedy !

Give sorrow words; the grief that doesnot speak,whisper the overwrought heart and bids it break-Shakespeare, Macbeth 5.1.50-1.