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Prenatal Cytogenetic Diagnosis in Couples with Bad Obstetric History - A Study of 1200 Cases

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Couples who have had two or more miscarriages are at increased risk of either of the partners carrying a structural chromosome abnormality. It is reported that the incidence of carrier status increases from approximately 0.7% in the general population to 2.2% after one miscarriage, 4.8% after two miscarriages, and 5.2% after three miscarriages. If one of the partners carries a structural chromosome abnormality, products of conception can have a normal karyotype, the same karyotype as the carrier parent, or an unbalanced karyotype. The presence of unbalanced karyotype can lead to miscarriage, stillbirth, or the birth of a child with major congenital anomalies. Prenatal diagnosis is therefore offered to carrier couples in subsequent pregnancies.
We present a study of 1200 couples referred to our center for chromosomal analysis with history of repeated pregnancy losses (more than twice), previous products of conception with chromosomal rearrangement and previous child with congenital malformations or mental subnormality.
Of the 1200 couples 98 had various chromosomal rearrangements. Of these 22 had various autosomal translocations (10 Robertsonian and 12 Reciprocal translocation), 34 had inversion of chromosome 9, 2 had 9qh+, 2 had 1qh+ and 22ps+, 28 had inversion of chromosome Y, 4 had deletion of chromosome Yq region, 4 had inversion of chromosome 3, 2 had inversion of chromosome 2.
All the couples were counseled for the consequences of their chromosomal rearrangement. Since inversions carry a low risk except chromosome 2 and 3 they did not opt for prenatal diagnosis. Of the 22 couples with autosomal translocations 20 under went cytogenetic diagnosis by CVS and amniocentesis. Various pregnancy outcome ranging from partial monosomies, trisomies and balanced translocations were observed and the same are discussed.

Molecular Analysis of Fetal RhD Status Using Cell Free DNA in Maternal Circulation

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Introduction: Cell free fetal DNA found in maternal plasma and serum is clinically very useful and has opened up new possibilities for non-invasive prenatal diagnosis. This application is useful for the investigation of sensitized RhD negative women whose partners are heterozygous for the RhD gene. As there is 50% chance of fetus being RhD positive and 50% chance of being RhD negative, the lady has to undergo repeated invasive testings to evaluate the extent of fetal hemolysis by optical densitometry of amniotic fluid or fetal blood sampling for serology. So to avoid fetomaternal risks associated with conventional invasive methods there is a need of a non- invasive procedure.
Methodology: In 2005 we have done 11 fetal RhD genotyping from maternal blood. 10ml EDTA blood samples were collected from 11 RhD negative pregnant women who were referred to us for amniotic fluid PCR to detect fetal RhD status at 26-34 weeks of gestation. Fetal DNA was isolated from plasma

of these women by Q1AMP DNA blood Midi kit. A nested PCR was done to amplify the 3'end of RhD gene. PCR products were analyzed by agarose gel electrophoresis. The results of Fetal RhD status obtained on amniotic fluid PCR and maternal blood was compared.
Results: We could isolate sufficient quantity of good quality DNA in all samples .The accuracy of the results was assessed by doing RhD genotyping from Amniotic fluid. Out of 11 fetuses 10 were corresponding with AF results but one showed false positive in maternal plasma.
Conclusion: The use of maternal DNA to establish RhD genotype of the fetuses can be introduced into current management of pregnancies with risk of Rh isoimmunization. This techniques may also have implication for the rational use of anti-D immunoprophylaxis by limiting its use only to RhD negative women carrying RhD positive babies.
Future Prospects: It is an ongoing process. By using improved methods like Real Time PCR the specificity of the test can be improved and it will also be possible to determine fetal genotype at earlier gestation. The same technique can be of use in other diseases also.

Prenatal Diagnosis of Cystic Fibrosis in India

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Cystic fibrosis (CF) is a life limiting, autosomal recessive, multi-system disease, which primarily affects the respiratory and gastrointestinal systems. Despite improvements, treatment has remained burdensome and expensive. Cure through gene therapy, which seemed possible after cloning of the putative CFTR gene in 1989, has still remained elusive. The only mode of prevention is through prenatal diagnosis of carrier couples and selective termination of affected fetuses.
Here, we present the data on prenatal diagnosis of CF carried in our centre between 1995 and 2005. Twenty-two prenatal diagnoses by chorionic villus sampling were done in families with an affected child, either through direct mutation testing or by linkage based approach. The affected child was homozygous for deltaF508 and both parents were heterozygous for deltaF508 in 10 families. In one family, mutation analysis was not carried out in the affected child and both parents were heterozygous for deltaF508. In one family affected child and the mother were heterozygous for deltaF508 and the father's mutation was not identified. In 9 families, in which mutation could not be identified, linkage based analysis was done using dinucleotide repeat markers in IVS8 and IVS17b.
Of these 22 prenatal diagnoses, 2 fetuses were affected and terminated. 20 were unaffected and the pregnancy was continued. 2 pregnancies are continuing and the rest 18 children from these pregnancies were healthy on follow up after delivery.
Thus, prenatal diagnosis of CF can reliably be carried out either by direct mutation testing or by linkage based approach and is of great help to the concerned families.

An Epidemiological Study of Congenital Malformations in Newborn

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Objective: To determine the prevalence of congenital malformations in neonates in Maulana Azad Medical College and Lok Nayak Hospital, New Delhi over a period of 3years and their antenatal associations and indicators.
Methods: The study included all live born and still born neonates born in Maulana Azad Medical College from Jan 2002 to Dec 2004. They were examined at birth to identify any congenital defects.

Photographs, radiographs, autopsy and chromosomal studies were undertaken when recommended. Malformations were classified into systems according to World Health Organization recommendations. Antenatal data from records included maternal age, parity, parental consanguinity; family history of congenital malformations, previous obstetric history and clinical course in present pregnancy.
Results: A total of 22,834 newborns (including 646 stillbirths) were delivered during the period of study. Out of these, 198 babies had one or more congenital malformations thus, making the overall prevalence of 0.87%. Amongst the 646 stillborns, 42 babies had some congenital malformation. Therefore the incidence of congenital malformations was much higher in stillborns (6.5%) as compared to the live born babies (0.7%). 21.2% of the live born babies died in the early neonatal period. The most common anomalies were of the central nervous system (35.8%) followed by the gastrointestinal system (16.7%) and the musculoskeletal system (10.1%). 2.5% cases had a history of affected relatives of the same or different condition.1.5% cases had parental consanguinity. 15.7% cases had history of previous abortions. Clinical indicators in the antenatal period like malpresentations (15.7%), polyhydramnios (14.1%), oligohydramnios (12.6%), IUGR (9.5%), placenta previa (4.5%) and twinning (3.5%) were present. 26.8% mothers had pre-term labour and (9.5%) had pre-eclampsia. Most (57.7%) of the congenital malformations were diagnosed in the antenatal period in those who had an ultrasound.
Conclusion: The prevalence of congenital malformations was 0.87%. Malformations of the central nervous system were the most common type of anomalies. Antenatally, congenital malformations are mostly associated with certain obstetrical clinical indicators and an abnormal ultrasound.

Genetic Counseling in Pregnancy – Experience at Sir Ganga Ram Hospital

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Introduction: Genetic counseling is increasingly being sought by couples planning a family, in view of the fast improving knowledge and awareness amongst the referring doctors and patients.
Material & Method: All patients referred to our center for genetic counseling for a period of one year (01 Jan '05 to 31st December, 05) were screened. Of these, only those seeking genetic counseling during pregnancy or related to pregnancy were enrolled.
Results: Total no. of patients counseled in year 2005 were 2125, of which 1286 (60.5%) patients sought advice for pregnancy. Among these, 1112 (87.0%) patients were pregnant, and 166 (13%) were referred for pre-pregnancy counseling. Indications for genetic counseling included abnormal triple test/ advanced maternal age (430/1286), abnormal antenatal ultrasound (188), previous child with Down syndrome/ chromosomal disease (74), a single gene disorder (184) or a metabolic disease (25). Other conditions included recurrent abortions/infertility (117), previous malformations (67), TORCH/Varicella infections (67), drugs during pregnancy (21), and miscellaneous conditions like maternal diabetes, hypothyroidism, non-specific mental retardation in previous child (113). About 18 patients had more than one reason for referral. Prenatal diagnosis for a specific condition was achieved by chromosomal, molecular, enzyme studies and ultrasonography in 763/1286.
Conclusion: Genetic counseling in pregnancy helps the couple to take an appropriate reproductive decision thus reducing the burden of genetic disorder in the family. We emphasize the need of early referrals and pre-pregnancy counseling in families at risk of a genetic disorder. This allows for timely prenatal diagnosis for proper management of the pregnancy.

Prenatal Diagnosis Using Amniotic Fluid Cultures - AIIMS Experience

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Amniotic fluid cultures for prenatal diagnosis of chromosomal anomalies was restarted in AIIMS about fifteen months back. During this period seventy-five amniotic fluid cultures were analyzed. The primary indications for PND were triple marker test being positive in 30.7% of the cases, 22.0% had ultrasound anomalies, 16.0% of the women warranted amniocentesis for having a previous child with Down syndrome. Advanced maternal age accounted for about 15% and other chromosomal abnormalities for about 9.3%. The mean age of the pregnant women was 32 years (range 20 - 44yrs) and the mean gestational age at the time of diagnosis was 19.0 weeks (16.3 – 21.2 wks). Prenatal diagnosis was performed on seventy-five pregnancies with a result of seventy-one normal Karyotypes, one case with trisomy 21, one carrier of Robertsonian translocation, and few with other chromosomal abnormalities. In two cases the patients required termination of pregnancy and one patient opted for termination. There were two IUDs and two neonatal deaths. The results will be discussed.

Lethal Skeletal Dysplasias – Ultrasound Correlation and Autopsy Diagnosis

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Lethal skeletal dysplasias (LSD) are a heterogeneous group of rare genetic disorders characterized by abnormal growth and development of bone and cartilage. We describe the diagnosis and outcome of 11 cases of lethal skeletal dysplasias evaluated between May 2003 and December 2005 for which autopsy data is available. Nine of these cases were identified by an abnormal antenatal ultrasound and two cases presented at birth. A final diagnosis was made on the basis of fetal autopsy, infantogram and molecular testing. The LSDs identified were Short Rib Polydactyly (2), Thanatophoric dysplasia (2), Osteogenesis Imperfecta, type II (1), Achondrogenesis (3) and Asphyxiating Thoracic Dystrophy(1). The cases diagnosed after birth were Campomelic dysplasia and Rhizomelic Chondrodysplasia Punctata.

Ultrasound findings of a lethal skeletal dysplasia were identified in all cases but in only 4 / 9 (44.4 %) was an accurate antenatal diagnosis possible. In 5 / 9 (45.5%) cases the autopsy data led to a diagnosis of a different type of skeletal dysplasia from the ultrasound diagnosis. In some cases there was a significant change in the risk of recurrence and genetic counseling.

Although careful sonographic examination may suggest the diagnosis, many cases remain undiagnosed. The presence of a narrow thorax suggests a bad prognosis. It is essential to obtain an autopsy, photographs, radiographs, karyotypic analysis, bone histopathology and molecular analysis in pregnancies terminated or at delivery. Conclusive diagnosis of the specific type of skeletal dysplasia is essential to establish the risk of recurrence and offer genetic counseling for future pregnancies.

Implication of Population Specific Median Value for Calculating MoM for Risk Prediction for Down's Syndrome

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Currently maternal serum screening for Down's syndrome using AFP, free beta HCG and unconjugated oestriol (uE3) is performed using several dedicated softwares. The analysis is done by matching the actual values with the referral median values for calculation of the risk. However, the median values of each parameter are not based on Indian population. Hence, an attempt has been made to use the median values of Indian population as well as to evaluate whether such alternation affect the out comes of the triple test. For this purpose, 2500 triple test results for Second trimester screening were taken into consideration. The median value for AFP, free beta HCG and unconjugated oestriol (uE3) were calculated as per NCCLS guidelines for each week. The respective MoM and risk is calculated for Down's syndrome using PRISCA software. The results are compared with the range provided by manufacturer. Interestingly it has been noticed that the risk calculation is being affected by the difference between the median values. Moreover, more patients show positivity (cut off risk 1 in 250) by using the established median value. Thus, there is a strong need to establish and use the median values for triple test parameters pertaining to Indian population to improve the detection rate by triple test.

Non Immune Hydrops Fetalis: Diagnosis and Genetic Counseling

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Hydrops fetalis is the accumulation of fluid in fetal soft tissues and serous cavities. It could be immune or non immune. With the availability of anti -D, non- immune hydrops fetalis accounts for about 90% of cases of hydrops fetalis. Non Immune Hydrops Fetalis (NIFH) is heterogenous and the causes could be due to fetal malformations (cardiovascular commonest), chromosomal abnormaities, hematological, skeletal dysplasia, infections, and metabolic disorders.

We have analyzed 23 cases of nonimmune hydrops. They comprised 16.4% of cases referred for fetal autopsy. Cystic hygroma was present in 9 cases out of which 6 had Turner phenotype, one had lethal multiple pterygium, one had associated cleft lip. Two cases had Pena Shokier phenotype, two cases had anemia and urorectal malformation was present in one case. Hypoplastic left heart was present in one. In nine cases the etiology could not be ascertained (38.2%). Three cases, which were referred to us, had recurrent hydrops. One of them was found to have lethal multiple pterygium and the cause could not be found in other two.
Hydrops fetalis is heterogenous. Apart from Rh D, immune causes can be due to rare blood group antigens such as anti C, anti Kell etc. Serological antibody testing can be done to diagnose them. All other cases should have thorough evaluation of fetus to look for any associated malformations such as cardiovascular malformations, skeletal dysplasia and multiple malformation syndromes by autopsy. Karyotype analysis, alpha thalassemia screening, congenital infections should be done in all cases. Cases of recurrent hydrops can be worked
Correct diagnosis helps in counseling of the couple and provides exact recurrence risk.

Prenatal Diagnosis by FISH – 634 Cases at Jaslok Hospital

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Fluorescence *in situ* hybridization (FISH) is now a well-established genetic diagnostic test. In prenatal diagnosis, it is used for the rapid detection of common aneuploidies. At Jaslok Hospital this technique was used on 634 samples over a period of 4 years. A majority (612) consisted of amniotic fluid, while 17 were of chorionic villi and 5 of cord blood. Reporting was usually done within a day after overnight hybridization. Aneuploidy was detected in 20 (3.15%) cases of which trisomy 21, was present in 18. We also detected 1 case of trisomy 18 and one of XXXY (90%)/ XXY (10%) moscaicism. Karyotyping was subsequently carried out in our laboratory or the referral labs. Occasionally samples were only tested for chromosomes 21 and 13 at a rate as low as Rs. 2,500 when karyotyping was unaffordable. The results were uninformative in only 3 cases (<0.5%) due to poor sample quality/quantity. We also tested fixed pellets from other labs if karyotyping failed. One of the problems occasionally (2.3% cases) faced was a suspicion of false positivity due to cross hybridization or widely split signals in about 5% cells, as mosaicism can never be ruled out..