

POSTER - GENETICS (GENP)

Utility of Fetal Autopsy in Prenatally Diagnosed Fetal Malformations for Genetic Counseling

Ashwin B Dalal, Sankar VH, Shubha R Phadke

Introduction: Congenital malformations are a common cause of perinatal deaths accounting for 10-15% of perinatal mortality in India. The recurrence risk of these disorders varies from negligibly low to 25% depending on the genetic disorder and its inheritance pattern. Although an anomaly scan at 18-20 weeks can give a fairly accurate diagnosis, examination of the terminated fetus for associated anomalies is essential to reach the etiological diagnosis and provide genetic counseling.

Materials and methods: One hundred and thirty four consecutive fetuses showing congenital malformations on ultrasonography were included in the study. Each fetus was examined according to a pre-designed protocol. This included a photograph, whole body radiograph, external and internal examination. Histopathological examination of the relevant tissue and chromosomal analysis was done when possible.

Results: Out of 134 fetuses with prenatally diagnosed malformations, fetal autopsy confirmed the findings in 132 (98.5 %) cases. The remaining two cases showed absence of renal aplasia which was suspected on prenatal ultrasound, but they had other associated malformations which were not detected on ultrasonography. Fetal autopsy provided additional findings in 77 (57.4 %) cases. In 24 (31 %) of these 77 cases, the additional findings led to revision of etiological diagnosis. This resulted in significant change in the risk of recurrence in subsequent pregnancies.

Conclusion: The study confirms utility of fetal autopsy for providing extra information after detection of a malformation on prenatal ultrasonography.

Prenatal Diagnosis of Familial Cyanotic Heart Disease Associated with a Familial Form of Microdeletion by Fish

H.M. Gawde, Z.M. Patel, M.I. Khatkhatay, Rashmi Adhia, Aruna D'Souza, Shiny Babu

Genetic Research Center, National Institute for Research in Reproductive Health,
Jehangir Merwanji Street, Parel, Mumbai – 400 012, Maharashtra, INDIA

Microdeletion of chromosome 22 is responsible for DiGeorge syndrome, Velo cardio facial syndrome and Conotruncal defects. We report our experience of prenatal diagnosis in a family who had lost two children with complex cyanotic heart disease. Fluorescence In Situ Hybridization (FISH) analysis in the couple revealed that the mother was mosaic for microdeletion of chromosome 22q11.2 in 10% of cells. Prenatal diagnosis was offered to her in her third pregnancy. On routine Ultrasonography at 16 weeks the four-chambered view of the heart was normal. However, before any further tests could be performed she aborted. FISH studies on the heart tissue of the abortus revealed 22q11.2 microdeletion with two different cell lines. Although chromosome 22q11.2 deletion has been documented postnatally prenatal diagnosis of this abnormality, particularly with mosaic form has not been reported. We describe here, what we believe to be the first case of prenatal diagnosis of mosaicism for a deletion 22q11.2 in a fetus, from India where routine sonography was apparently normal.

This suggests the importance of performing FISH studies when there is a history of congenital heart disease, even though Ultrasonography showed a normal four-chambered view of the heart.

Duchenne Muscular Dystrophy Methods of Risk Assessment and Prenatal Diagnosis in Susceptible Families

Hema Purandarey, Prabodh Mallick, Vishalakshi Mamath

Center for Genetic Health Care, A-11, Elco Arcade, Hill Road, Bandra (W), Mumbai – 50
Tel : 022 26420428 / 26400118, Email : medgen@giasbm01.vsnl.net.in

Duchenne Muscular Dystrophy (DMD), the most severe form of muscular dystrophy, is an inherited neuromuscular disorder with physical and often intellectual implications. DMD is caused by a mutation in the dystrophin gene located on the short arm of the 'X' chromosome at p21.1 position. The most remarkable feature of the DMD gene is its size estimated to be 2300 KB or 1.5% of the 'X' chromosome. It is transmitted as "X-Linked" recessive inheritance with the incidence of about 1 : 3500 live male births. About 2/3 of the cases are new mutations, while 1/3 are inherited from carrier mothers. In rare instances, females have been reported with DMD, some have X; autosome translocations, others have only one 'X' chromosome with a DMD mutation on that chromosome and a rare group consists of heterozygous monozygotic twins. In non-carrier females possible explanation is it can be due to fresh mutation or gonadal mosaicism.

In order to offer recurrence risk estimation and prenatal diagnosis in high-risk pregnancies a protocol is essential. This includes detailed pedigree analysis, and conventional cytogenetic analysis in the female to note any chromosomal rearrangement to assess risk to female offspring, molecular method for detection of any deletions in the affected and carrier status in a female.

Prenatal Diagnosis of Spinal Muscular Atrophy

Sadhna Arora, Madhulika Kabra, Sheffali Gulati, Deepika Deka, Alka Kriplani, Veena Kalra, Suneeta Mittal, MC Sharma, Chitra Sarkar

Genetic Unit, Department of Pediatrics, AIIMS

Introduction-Spinal Muscular Atrophy (SMA) is an incurable and most common inherited lower motor neuron disease. It is an Autosomal recessive disorder characterized by the degeneration of anterior horn cells of the spinal cord. Type I, II and III are the three types, which have been described according to decreasing severity. SMN & NAIP are the two candidate gene implicated. Deletion in exon 7 of SMN 1 gene harbored by chromosome 5 is positive in 90% of the patient according to Western literature.

Methods-Over the past 8 years we have provided prenatal diagnosis to 90 pregnant women for SMA. 74 (82.2%) women already had one or more affected children with suggestive clinical features and positive muscle biopsy / or EMG and 16 (17.77%) women had children who died of symptoms suggestive of SMA.

Exon 7 deletion was screened first in affected child, if positive prenatal diagnosis was offered. Homozygous deletion of SMN 1 gene was tested by using PCR followed by restriction with Dra 1 and 3% agarose gel electrophoresis.

Results: Out of 90 pregnancies 65 (72.22%) fetuses were deletion negative and 25 (27.77%) were deletion positive. Children born after normal report were followed in genetic clinic and were asymptomatic. No false-positive or false-negative results on prenatal testing were found.

Conclusion: We conclude that PCR-RFLP based technique for molecular diagnosis of SMA is simple and reliable.

Recurrence of Esophageal Atresia with Tracheoesophageal Fistula in Consequent Pregnancy with Co-existence of Defect in Twins

Basil Lyngdoh, Suman Lal, Alka Kriplani, Meenakshi Sharma, Nutan Agarwal, Neerja Bhatla
 Department of Obs & Gynae, AIIMS
 E-mail: cityrama@yahoo.com

Introduction: Esophageal atresia with tracheoesophageal fistula is an uncommon fetal malformation occurring one in 3000 to 5000 births. Though the etiology is not known and only isolated familial cases have been reported¹. We report a case of twins both having esophageal atresia with tracheoesophageal fistula with history of a similar defect in the previous child.

Case-report: A 20 year old G2P1 registered in our high risk antenatal clinic had a diamniotic dichorionic twin pregnancy and a level two ultrasonography revealed that both twins had a single umbilical artery. However no other anomaly was detected. Patient developed polyhydramnios in both amniotic cavities at 28 weeks period of gestation and had a preterm vaginal delivery at 33 weeks. Two male babies were born weighing 1.68kg and 1.62kg respectively. The placenta was diamniotic dichorionic and both the cords revealed single umbilical artery. A diagnosis of tracheoesophageal fistula and esophageal atresia was made in both twins soon after birth when there was excessive oral secretions and the orogastric tube could not be passed beyond 10 -12 cms. Both babies underwent a right thoracotomy with fistula ligation and primary resection-anastomosis of the atretic portion. The babies had an uneventful post operative course and were discharged in a healthy condition.

Discussion: The recurrence of esophageal atresia with tracheo-esophageal fistula in subsequent pregnancy is a rare occurrence. In a population based study of esophageal atresia with tracheo-esophageal fistula only one case out of 204 had a familial occurrence. It is more likely to occur in males with a male to female ratio of 1.44. The malformation is associated with a higher incidence of multiple birth (4.9%) and 17.6% of the affected infants have a single umbilical artery. Development of polyhydramnios, and inability to identify a fetal stomach bubble on ultrasonography, raises the suspicion of this malformation. However, definite diagnosis is usually missed till delivery.

Conclusion: In women with previous baby affected with esophageal atresia and tracheoesophageal fistula perinatal outcome can be improved by proper prenatal counseling with serial ultrasounds to detect the malformation and delivery at a tertiary care centre.

Prenatal Diagnosis of Incontinentia Pigmenti

Seema Thakur, Ratna D. Puri, Sunita Bijarnia, Renu Saxena, Michael A. Patton, I C Verma
 Department of Genetic Medicine, Sir Ganga Ram Hospital New Delhi, St. George's Hospital Medical School, London

Incontinentia Pigmenti is a disorder characterized by abnormal skin pigmentation, anodontia, nail dystrophy, alopecia, retinal detachment and central nervous system defects. It is X-linked dominant disorder which is lethal in males. Affected females transmit the disease to 50% of their sons or daughters.

It is caused by mutation in a NEMO gene and 80% are due to a common deletion.

We present a family who came to us at 7 weeks for prenatal diagnosis of incontinentia pigmenti. The affected female (12 years old) was short and had classical features of incontinentia pigmenti in skin, 'teeth, hairs, nails and eyes. Her mother also had partially absent teeth and was short. She did not have typical skin lesions. Her grandmother was also short and has partially absent teeth. Her grandmother's sister had death of two male fetuses at 24 hours and 6 hours of life.

We tested the affected child and her mother for the presence of deletion of NEMO gene by long range

PCR. Deletion of NEMO gene was present in both of them. Prenatal diagnosis was possible at 14 weeks by analyzing chorionic villi sample for deletion in NEMO gene. DNA analysis showed that the fetus was unaffected.

PND of Large Centromeric Heteromorphism of Chromosome 15 - Implications for Genetic Counseling

A. Radha Rama Devi, P. Rajitha, Suma Prasad, K. Jayaram
Head, Diagnostic Division, CDFD, 7-18, Nacharam, Hyderabad
E-mail: radha@cdfd.org.in

Centromeric variations in amniocytes during PND can create difficulties in genetic counseling. We present a case of PND in a second gravida with positive second trimester triple marker screening for Down syndrome with 15p+ suggestive of 15:21 translocation. FISH was performed on metaphase chromosomes from this patient using LSI13/21 and LSI D15S11/CEP15(G) probe, localized to 13q14(G)/21q22.13-q22.2(R) and 15q11-q13(R) region. Probe hybridization with LSI13/21 showed two green and two red signals as expected for chromosome 13 and 21. Probe D15S11/CEP15 showed two red signals at 15q11-q13 region for D15S11 as expected, CEP 15 also showed two green signals, but one of the green signals for CEP 15 is of double the size than the other, in all the metaphases analysed. This indicates a heteromorphic duplication of the centromeric region of chromosome 15. The study implies that heteromorphic duplications are normal variants containing repetitive sequences of the DNA and has no significant impact on the phenotype. FISH can differentiate the suspected translocation observed in the G-banded amniocytes showing the power of FISH in the PND making genetic counseling easy and appropriate. In the absence of FISH, counseling would have been different.

Prenatal Genetic Counseling for Chromosomal Abnormalities

Preetha Tilak, Rema Devi
Division of Human Genetics, St. John's Medical College, Bangalore – 560 034

Chromosomal abnormalities are present in 10% of spermatozoa and 25% of mature oocytes. Incidence of spontaneous miscarriage in all recognized pregnancies is to the tune of 15-25%. Conversely, 50% of spontaneous miscarriages and 20% of the 'morphologically normal' embryos have a chromosomal abnormality. The magnitude of chromosomal abnormality being pretty high warrants the need for genetic counseling and prenatal genetic diagnosis. The Division of Human Genetics (DHG) in St. John's Medical College, Bangalore is a referral center for Karyotyping and genetic counseling given to some of the cases with multiple congenital anomalies, mental retardation and bad obstetric history referred to DHG for Karyotyping and where chromosomal abnormality was detected.

Molecular Diagnosis Assisted Preconception Counseling in a Family with Lissencephaly Child

Vineet Bhusha Gupta¹, Sohani Verma², Vimarsh Raina^{3*}, Iravathy Goud³

1. Department of Neurology, Indraprastha Apollo Hospitals, New Delhi – 110 044

2. Department of Gynecology & Obstetrics, Indraprastha Apollo Hospitals, New Delhi – 110 044

3. Molecular Biology & Immunology Lab, Indraprastha Apollo Hospitals, New Delhi – 110 044

Background: Lissencephaly is a clinically and genetically heterogeneous malformation of the brain, leading to a severe disabling condition and seizures. The recent discovery of molecular techniques and identification of lissencephaly genes (LIS1 and DCX) has allowed etiologic diagnosis of this disorder feasible.

Objective : To describe a patient with lissencephaly in whom fluorescence in situ hybridization (FISH) and DCX mutation analysis determined etiologic diagnosis, providing precise genetic counseling and possible prenatal diagnosis for the family.

Clinical Report & Study Results : The authors report a 4-year boy who presented with status epilepticus triggered by fever at the age of 3 years. He had a history of recurrent seizures in the past. He had delayed milestones, partial neck holding and no development of speech. He is unable to recognize his parents. The patient was born as a full term forceps delivery to a G2P1A1 29-year-old woman. Chromosomal analysis of peripheral blood of the parents and the child revealed normal karyotype. Computed tomographic scan of the brain showed a total absence of gyral formation. FISH of the metaphase chromosome from the patient, using Miller-Dieker/LIS1 (17p13.3) and control probes D17Z1 showed two signals Miller-Dieker/LIS1 probe, indicating no microdeletion of 17p13.3 region including LIS1 gene and a control signal in the child and the parents. The DCX gene mutation was also performed in the parents and the child, which showed mutation of the DCX gene in the child and not in the parents.

Conclusion : A confirmation of DCX gene mutation in this patient leads to an etiologic diagnosis of lissencephaly. This information allowed precise genetic counseling, estimation of recurrent risk, and definite prenatal diagnosis available to the family and also further suggests that FISH – 17p13.3 and DCX mutation analysis studies should be performed in addition to a standard metaphase analysis in all patients with type I lissencephaly.

Spectrum of Renal Malformation at Autopsy

Sunita Bijarnia, Seema Thakur, Ratna D. Puri, , Meena Lal, Renu Saxena, Subimal Roy*, I C Verma
Department of Genetic Medicine, Pathology* Sir Ganga Ram Hospital New Delhi.

Antenatal detection of renal malformation on ultrasound are common (1 in 300). These malformations may be lethal, semilethal or soft marks on ultrasound. We present a 3-year data of renal malformations referred for autopsy after detection of oligohydramnios on ultrasound. . Total no. of autopsy done during this period was 140 out of which 21 had renal malformations. (15%). Isolated renal anomaly was seen in 15 cases whereas 6 fetuses had multiple malformations. Bilateral multicystic dysplastic kidney was the most common (4/15). Posterior urethral valve and urorectal malformations were present in 14% each. The other malformations detected were hypoplastic kidney, bladder exstrophy, polycystic kidney, pelviureteric junction obstruction . One fetus had an ureteric cyst along with urethral atresia. 3 cases with multiple malformation had Meckel Gruber syndrome(14%), 2 fetuses had Turner phenotype and one had Fryns syndrome. Exact etiological diagnosis helps in calculating the recurrence risk for the families and it also gives the assurance that most of them are sporadic and can be diagnosed on ultrasound at 16-18 weeks.

Prenatal Diagnosis of Hemoglobinopathies- Experience at a Tertiary Care Center

Madhulika Kabra, Sadhna Arora, Shivram S Shastri, Manju Ghosh, G.PDas, Neerja Gupta, Deepika Deka*, Alka Kriplani*, Suneeta Mittal *

Genetics Unit department of Pediatrics & Department of Obstetrics and Gynecology
All India Institute of Medical Sciences New Delhi

Introduction: In our country of limited resources 30 million carriers of b-thalassemia produce 7500 infants annually with a hemoglobinopathy. These hemoglobin disorders include homozygous b-thalassemia, HbE/b-Thalassemia, HbS/b-Thalassemia, HbSS, HbD/b-Thalassemia and homozygous HbD etc. The phenotype may vary from a severe anemia with transfusion dependency to a totally asymptomatic individual. Management of these severe hemoglobinopathies is very expensive. So to

reduce the burden on these families and national health services genetic counseling and prenatal diagnosis in combination is the preferred way.

Methods- Over the past 8 years we have provided prenatal diagnosis (PND) for 780 pregnant women at risk of having a baby with a hemoglobinopathy. These included 747 (95.76%) for β -thalassemia, 18 (2.3%) for HbE/ β -thalassemia, 10 (1.28%) for HbSS or HbS/ β -thalassemia and 5 (0.64%) for homozygous HbD or HbD/ β -thalassemia. Strategy for prenatal diagnosis included DNA extraction from confirmed carriers followed by mutation detection using Amplification Refractory Mutation System (ARMS) in parents and then in Chorionic Villus Sample (CVS). Restriction fragment Length Polymorphism (RFLP) was done where mutation was not characterized in one or both of the parents and prenatal diagnosis was offered if 2-3 markers were informative.

Results- A confirmed diagnosis was given in 759 (97.3%) pregnancies. Out of 780 pregnancies 397 (50.89%) were reported as carriers, 208 (26.66%) as normal, 154 (19.74%) as affected. Remaining 21 (2.6%) were reported either carrier or normal as mutation was characterized in one of the parent and it was not inherited by the fetus. Maternal contamination was ruled out by using VNTR, like apoB wherever was necessary. On follow up after delivery all children who were reported normal / carrier were healthy.

Conclusion- Simple PCR based methods like ARMs and RFLP can effectively be used for PND of thalassemia, thus reducing a major burden on the families at risk and the society.

Prenatal Diagnosis of a 2n/4n Mixoploid Fetus with Severe Hydrops

Arundhati Sharma*, **Vatsala Dhadwal***, **Yutika Sharma***, **Deepika Deka***, **Kiran Kucheria***

*Department of Anatomy, AIIMS, New Delhi, India.

*Department of Obstetrics and Gynecology, AIIMS, New Delhi, India.

Tetraploidy is characterized by four complete sets of chromosomes ($4n=92$). It is frequently reported in Spontaneous Abortions and is extremely rare in term pregnancies. Most of the late surviving patients are diploid-tetraploid mosaics or mixoploids. Tetraploidy occurs frequently in amniotic cells and is considered a culture artifact wherein the fetus is chromosomally normal. But on rare occasions the presence of Tetraploidy indicates a fetus which is chromosomally abnormal or with multiple congenital defects. We report here a case of fetal hydrops with diploid/tetraploid mosaicism found in amniocytes, which was confirmed later in fetal cord blood. The karyotyping of the parents was also done as the patient had delivered previously two macerated fetuses and one preterm hydrops baby. The father had a normal chromosome complement but the mother showed a low-level diploid/tetraploid mosaicism. They had one normal daughter.

The diagnosis of mixoploidy is important for purposes of accurate genetic counseling and such findings are useful in guiding the clinicians and geneticists to not uniformly dismiss tetraploidy as artifactual in amniocytes but to confirm its presence in other fetal tissues in cases with ultrasound abnormalities.

Ethical Implications for Prenatal Diagnosis Among Indian Cases with Non-syndromic Recessive Deafness

Manju Ghosh, **G.P. Das**, **Vijaya R**, **Manjula Maheshwari**, **Madhulika Kabra**

Genetic Unit, AIIMS

Non-syndromic Recessive Deafness (NSRD) which constitutes 80% of congenital deafness has taken the central stage of the research on deafness. Of the multiple number of genes that are responsible for

NSRD the contribution of Connexin 26 was found to vary from 10% in Taiwan to 88% in Ashkenazi Jews. With this back ground in mind a screening program was initiated to screen four common mutations of the gene in Indian cases on randomized basis.

Sixty six families with NSRD were screened for four common mutations viz W24X, W77X, Q124X and 35delG. Of these families there were 5 families who had 3 or more cases affected with NSRD, 21 cases with 2 affected and 40 with 1 affected and there by a total of 189 affected cases were subjected to screening for these mutations. Seven families (10.6%) were detected to have the mutation.

The attitude and perceptions of the parents towards prenatal diagnosis has been constantly evolving among Indian population. Although the parents fully understand that the disorder is not lethal they are keen to opt for prenatal diagnosis agreeing to terminate the pregnancy if the result is positive. The deaf community in USA are against the prenatal diagnosis and are raising ethical issues on such modalities of management. The prenatal diagnosis in these cases is yet to be formally accepted by the scientific community. The scenario in our country is different due to paucity of facilities for rehabilitation of deaf individuals. The issue of prenatal diagnosis should be discussed.

Spectrum of Congenital Anomalies in a Tertiary Care Centre in Uttaranchal

Savita Bansal, Babita Nandal, Vineeta Gupta, S.K. Mudgil

Department of Obstetrics & Gynaecology, Himalayan Institute of Medical Sciences, Jolly Grant, Dehradun.

We analyzed 97 cases of congenital anomalies detected at birth or referred from outside in Himalayan Institute of Medical Sciences, Jolly Grant, Dehradun. We compared our data with available data from North India.

Most common congenital anomaly was neural tube defect (23.7%), followed by trecheoesophageal fistula (11.3%), Congenital Heart disease (11.3%), Imperferoate anus (6.18%), Cleft lip Palate (5.15%), hydrocephatus (4.12%), Omphalocele (3.09%) etc. Detection of most of congenital anomalies was late in IInd trimester, IIIrd trimester and after delivery. Neural tube defects were also detected after 24 weeks where decision of termination of pregnancy becomes difficult.

Conclusion: Therefore there is need for universal biochemical screening of all pregnancies for NTD especially in North India. Strategies should be made to detect structural anomalies before 20 weeks.

Cytogenetic and Molecular Analysis in Cases with Hypogonadism and Variant Klinefelter Syndrome

Rima Dada, R Talwar, K Kucheria

Dept. of Anatomy, All India Institute of Medical Sciences, New Delhi-110029, India

Klinefelter Syndrome (KFS) is the commonest sex chromosomal abnormality and the commonest cause of male infertility. About 12-15% cases are mosaics with variable phenotype. Most reports do not differentiate KFS from the variants. These variant cases have additional phenotypic anomalies and thus form a distinct entity and therefore were studied in detail. In the present study 145 cases of male infertility were analysed cytogenetically. Twenty-five well spread G banded metaphases were karyotyped using image analyser (Cytovision, Applied Imaging). In mosaic cases 50 metaphases were analysed. Six mosaic variant cases with more than one cell line were analysed at the molecular level by Fluorescence In situ Hybridization (FISH) to detect low level cryptic mosaicism. Semen analysis was done according to the WHO guidelines (1999). We found 11cases with KFS, nine cases were KF mosaics and 5 were mosaic variants. These variant mosaic cases were Case1- 47,XXY (60%)/48,XXYY (16%)/46,XX (20%)/47,XY (4%), Case 2- 47,XXY (91%)/48,XXYY (3%)/48,XXYY (3%)/46,XY (3%), Case 3- 47,XXY (60%)/48,XXYY (26%)/49,XXYY (14%), Case 4- 46,XY (50%)/47,XXY (30%)/48,XXYY

(20%), Case 5- 46,XY (53%)/48,XXXY (40%)/49,XXXYY (7%) case 6- 47,XXY/48,XXXY. FISH detected an additional cell line of 50,XXXXYY (1%) in case1. These variant cases had additional features than of KFS like mental retardation, difficulty in expressive language, slurred speech, Mitral Valve Prolapse and adjustment problem with peers. FSH and LH were markedly elevated and Testosterone levels were low. All cases were azoospermic. FISH as an adjunct to conventional cytogenetics helps in identifying cell lines not identified cytogenetically. Thus variant cases should be considered as a distinct entity as they have additional cardiovascular and other clinical features and should thus be managed accordingly.

Genetic Analysis of EB Patients of Intermediate Phenotype

Vandana Arya, Mandakini Pradhan, Usha R Gupta, Sarita Agarwal

Department of Genetics, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow (U.P.) - 226014.

A Thalassaemia Intermedia is the clinical definition used for defining varied clinical manifestation of thalassaemia disease ranging in severity from the asymptomatic carrier state to the transfusion dependent thalassaemia major. The mild phenotype may result from homozygosity for mild β thalassaemia mutation, compound heterozygous for mild and severe β thalassaemia mutations, coinheritance with homozygous β thalassaemia and a thalassaemia or hereditary persistence of fetal hemoglobin, double heterozygosity for β thalassaemia and triple alpha globin arrangement or the presence of the highly unstable hemoglobin variant. Herein, we report 12 unrelated individuals (5 -35 years) belonging to U.P. descent, with the clinical phenotype of thalassaemia intermedia in whom ARMS _ PCR analysis of Beta globin gene reveals one β globin gene mutation on one chromosome and HbE (CO -26 Glutamine-Lysine) mutation on the other chromosome. To explain further the thalassaemia intermedia phenotype, we dissected the type of β thalassaemia mutation and found 7/12 (50%) IVS 1-5 G-C (β^+ type), two chromosomes of β^0 type; one each of CO30(G-C) and CO 41/42 (-CTTT), while, on the two chromosome the mutation was on β^{++} type related to -88 (C-T) and Cap+1 (A-C). Gap PCR for alpha globin gene arrangement reveals no deletion or triplication in any of the samples, however, PCR -RFLP analysis indicates presence of Xmn 1 polymorphism in 9 alleles (37%) and absence (63%) in 15 alleles. Two cases where HbF is below 1% that can be correlated well with complete absence of Xmn 1 site (-/-) associated with milder mutations of β thalassaemia. The level of HbF in 6 subjects' ranges from 18.9 - 41.1% is associated with Xmn1 site (+/-) polymorphism. The present paper discussed the variability of phenotypes of E β thalassaemia patients like onset of the disease, number of blood transfusion, liver and spleen sizes etc and molecular genotyping.

Ignorance About Genetic Diseases Among Rural Population: A Challenge

Tripti Singh, Anjan J. Talukdar, Deepika Deka

Dept. of Obs & Gynae, AIIMS, New Delhi

We are at a time of unprecedented increase in knowledge and of rapidly changing technology. A World Health Organization expert consultation concluded that "Genetics advances will only be acceptable if their application is carried out with due regard to autonomy, justice, education, beliefs and resources of each nation and community." Public health authorities are increasingly concerned by the high rate of births with genetic disorders especially in developing countries. OBJECTIVES To assess the awareness about genetic diseases and congenital malformations among general population in rural setup in India. METHODS We conducted a cross-sectional survey and used the method of stratified random sampling. We interviewed 100 people according to a pre structured questionnaire. RESULTS There is negligible awareness about genetic diseases among general population. Hence, there is a need for educational programs for the general population. The primary level of care should

be the basis of all health actions in genetics, with emphasis on programmes that use simple, affordable technology and reach a large proportion of the community. Examples of actions at the primary health-care level include public education in genetics, detection of genetic risks in the community through due attention to and recording of family history in all patient encounters with the health system and encouragement of reproduction at optimal maternal ages.

Genetic Diseases and Congenital Malformations- Are Primary Care Physicians Aware?

Anjan J.Talukdar, Tripti Singh , Deepika Deka
Dept. of Obs & Gynae, AIIMS

In the 21st century, we, in the developing nations, are alarmed by the high rate of births with genetic disorders. This trend can be due to ignorance, natural selection or some unknown factor. So in order to find out we conducted a study. **OBJECTIVE:** To assess the awareness about genetic diseases and congenital malformations among doctors in rural setup in India. **METHODOLOGY:** We conducted a cross-sectional survey and used the method of stratified random sampling. We interviewed 50 doctors according to a pre structured questionnaire. **RESULT:** Some of the doctors have knowledge about genetic diseases and congenital malformations but none of them are implementing their knowledge in practice.

“India is a country of vast diversity”. This saying is true not only for the cultural and religious practices but also for medical practices. At one end we are conducting conferences on IVF and Fetal medicine but the doctors who serve the bulk of the population are faintly aware of common genetic diseases prevalent in the country. Hence, there is a need for educational programs, for the primary health care physicians, to be organized on a regular basis.

Chromosomal Aberrations and Its Association with Reproductive Failure

R Kumar, Rima Dada, Rajeev Kumar, Kiran Kucheria
Dept of Anatomy& Urology, All India Institute of Medical Sciences, New Delhi, India

Chromosomal abnormalities and aneuploidies are found to be associated with and have a higher prevalence in infertile males than in the general population. These aberrations not only result in partial or complete spermatogenic arrest but may also result in implantation failure and consequently failure of *InVitro fertilization* (IVF). Assisted Reproductive Technology (ART) has revolutionised the management of infertility and allows infertile couples to procreate.

Cytogenetic and molecular analysis was done in 165 infertile males and 30 couples going in for IVF. Twenty five well spread G banded metaphases were karyotyped using image analyser (Cytovision, Applied Imaging). In mosaic cases 50 metaphases were analysed. Chromosomal abnormalities were found in 46 infertile males. We found 16 cases with Klinefelter Syndrome (KFS), 20 cases were KF mosaics and 6 were mosaic variants, three cases with 46,XY 1qh+ and one case with 46,XY16h+. In 5 of the 30 couples couples opting for assisted reproduction cytogenetic analysis in the female partner revealed 46,XXq- chromosomal complement in two cases and Yq microdeletion in the AZF region in 2 cases. The AZF loci deleted was AZFc. Deletion of long arm of X chromosome(Xq-) in the female partner in two cases might have resulted in repeated failure of blastocyst development. This couple had gone in for 4 IVF cycles which had failed due to failure of blastocyst development. The male partner was cytogenetically normal and also had no Yq microdeletion spanning the AZF loci. In cases with sex chromosomal and autosomal aberrations there is probability of poor embryo development and consequently poor implantation, which may be a result of high segregation abnormalities and

may negatively affect the outcome of assisted reproductive techniques. Thus these infertile couples should be counseled prior to going in for ART about the importance of genetic analysis, and how the presence of genetic anomalies results in poor IVF outcome and vertical iatrogenic transmission of these anomalies to the offspring born through ART.

A Clinical Study of Birth Defects

G.C. Das, Monomita Chakravarty, Ranima Deka
Obs. & Gynae., GMCH

Objective: A clinical study of birth defects to determine the incidence, prevalence and various types of congenital anomalies in O & G Deptt, GMCH.

Method: A simple retrospective study using data from birth register of O & G deptt from the period of June 1, 2004 to December 31, 2005.

Subjects: Total 5324 emergency patients attending L/R O&G, GMCH & 72 newborns delivered with birth defects.

Results: The overall incidence of congenital anomaly in our institution was 1.35% with highest being NTD (27 Nos) – 0.30% followed by musculoskeletal defects (6 Nos) – 0.11%. Others were (23 Nos) – 0.43%.

Conclusion: Congenital malformed newborns are social stigmata thus reducing the incidence and prevalence of birth defects leading to the infant morbidity and mortality is an attainable goal. This is targeted to primary preventions by dietary supplementation, social awareness, prenatal diagnosis and subsequent termination of affected pregnancies. After all, motives of all doctors are *HEALTHY MOTHER WITH A HEALTHY BABY*.

Prenatal Diagnosis of Hemophilia A and Hemophilia B Using Factor VIII and IX Gene Polymorphism on Chorionic Villi - Indian Experience

Madhumita Roy Chowdhury, Neerja Gupta, Manju Ghosh, Madhulika Kabra, PSN Menon, Renu Saxena *

Genetics Unit, Department of Pediatrics & Department of Hematology*, AIIMS, New Delhi

Hemophilia A and B are X linked recessive debilitating coagulation defects. The heterogeneous nature of the mutations, size, and the complexity of factor VIII and IX gene makes direct mutation analysis difficult. Thus, carrier screening and prenatal diagnosis of hemophilia A and B is largely dependent on haplotype analysis by using restriction fragment length polymorphism (RFLP) and short tandem repeat (STR) markers to track the defective gene within a family. The main objective of the study was to assess the utility of various polymorphic markers for factor VIII (4 intragenic STR markers Hind III, Bcl I, Intron 13, introns 22; one extragenic St 14 marker) and IX gene (2 intragenic RFLP marker Dde I, Xmn I; 1 extragenic Hha I) in prenatal diagnosis. Sixty six hemophilia A and 23 hemophilia B chorionic villi samples over a period of 8 years from 1997 to 2005 were studied. Samples were first analyzed for determination of sex by using amelogenin markers followed by linkage analysis on male samples. Combined informativeness of these PCR based polymorphic markers is about 88% for factor VIII gene and 80% for factor IX gene in Indian population. This study further reiterates the usefulness of linkage analysis in prenatal diagnosis for genetic disorders with marked genetic heterogeneity and technically difficult direct mutation testing.

Chromosomal Analysis in Mental Retarded Children Born with Dysmorphic Features

Gurjit Kaur¹, Soumya Mishra, Alka Sehgal², B.S. Chavan³

1- Dept of Physiology / Genetic Centre; 2- Dept of Obstetrics & Gynecology; 3-Dept of Psychiatry, Govt Medical College, Chandigarh.

Email: gurjitkaur123@rediffmail.com

We have done karyotyping of 20 mentally retarded children, referred from mental retardation centre, GIMRC, Chandigarh, out of which five cases were diagnosed for trisomy 21, one child with mosaicism, and one child with Edward's syndrome. Rest of the children was found to have normal karyotype, indicating other biochemical metabolic defects. In addition, ten cases of Down's syndrome with trisomy 21 chromosome has been confirmed by karyotyping, referred from the Pediatric Department, with mongoloid features, muscular hypotonia, brachycephaly, protruding tongue, small low set ears, upward sloping palpebral fissures, single palmar crease, flat nasal bridge. It is noteworthy here that one case of Klinefelter's syndrome with 47, XXY karyotype is also observed out of five children having dysmorphic features at birth. The affected individual is 18 days old baby with the features of low set ears, malformed right-sided pinna, high arched palate, abnormal cry, wide spaced nipple, tuft of hair at sacral region, sandal gap in right foot, short neck and ambiguous genitalia.

Karyotyping of at Risk Fetuses by Cordocentesis

Madhulika Kabra, Rashmi Shukla, Vinod Kumar, Prahalad Balakrishnan, Deepka Deka[#], Alka Kriplani[#], PSN Menon, IC Verma^{*}

Genetic Unit, Department of Pediatrics, All India Institute of Medical Sciences, New Delhi; [#]Department of Obstetrics and Gynecology, All India Institute of Medical Sciences, New Delhi; ^{*} Department of Genetic Medicine, Sir Gangaram Hospital, New Delhi

Prenatal karyotyping using lymphocytes from cord blood is a useful method of detecting chromosomal errors in the foetus.

Cordocentesis was performed on 435 pregnant women between 18 to 40 years of age, for prenatal karyotyping, from January 1990 through August 2005. The gestational age of these women ranged from 18 to 38 weeks. The most common indication for cordocentesis was ultrasonographic abnormality (62.3%), followed by history of a previous child with Down syndrome (14.16%). Other indications included, advanced maternal age (7.62%), an abnormal triple screen (7.08%) or a previous child with a other malformations (7.35%). In only 1.35% cases, one of the partners was a balanced translocation carrier.

Analysis of 337 successful cultures showed 23 (7.82%) abnormal karyotypes. 43 samples could not be reported upon due to maternal blood contamination. In case of an abnormal karyotype, the couple was counseled and obstetric management was altered appropriately.

Importance and Awareness of Prenatal Diagnosis in the Indian Context

Arundhati Sharma^{*}, Sethi R^{*}, Kabra M[#], Kriplani A^{\$}, Kucheria K^{*}

^{*}Department of Anatomy, AIIMS, New Delhi

^{\$}Department of Obstetrics and Gynecology, AIIMS, New Delhi

[#]Department of Pediatrics, AIIMS, New Delhi

Prenatal testing guidelines recommend offering amniocentesis or CVS sampling to women aged 35 years or who have been found by screening to be at risk of giving birth to an infant with any chromosomal abnormality. We report here a case of a woman, gravida five, aged 32 years referred to us for Genetic amniocentesis at 21.5 weeks of gestation. The patient had one twelve-year-old daughter with Down syndrome and one six-year-old normal daughter. She had two spontaneous abortions at about two month's gestation. She was not offered maternal serum or ultrasound screening but was referred to AIIMS from an adjacent city at about 20 weeks gestation. Cytogenetic analysis was carried out on amniotic fluid, which showed mosaic trisomy 21. The results were confirmed by fluorescence in-situ hybridization (FISH). The patient and her husband were counseled and they opted for termination. The pregnancy was terminated and the cord blood was karyotyped. We found again the presence of mosaicism for trisomy 21 confirming our earlier results. This shows the importance of prenatal testing and the need for awareness regarding the availability of such tests among the clinicians and others who are engaged in the care and management of such patients. At the same time maternal serum screening should be offered to all the women irrespective of their age. Women in the high-risk group should be evaluated for maternal age, biochemical and USG markers which together should be used to screen for chromosomal defects.

Prenatal Diagnosis in Achondroplasia

Madhumita Roy Chowdhury, Deepka Deka[#], Alka Kriplani[#], Madhulika Kabra

Genetic Unit, Department of Pediatrics, All India Institute of Medical Sciences, New Delhi, [#]Department of Obstetrics and Gynecology, All India Institute of Medical Sciences, New Delhi;

The phenotypic variation of skeletal dysplasias point to a complex etiology for this class of disorders. Some of the more common skeletal dysplasia however, have been shown to be a consequence of a limited number of mutations in the fibroblast growth receptor2 (FGFR 3) gene.

Achondroplasia is the most common form of autosomal dominant inherited dwarfism. Its incidence has been estimated to range from 1/15000 to 1/77000. If either of the parents is affected the risk of recurrence is 50% in subsequent pregnancy. If parents are normal, the risk of recurrence is very low. The gene has been mapped to 4p16.3 and eventually shown to result from distinct mutation in the fibroblast growth factor receptor gene, FGFR 3. The most common mutation results in specific glycine to arginine substitution at amino acid 380 of FGFR 3. This non-conservative change is due either to nucleotide 1138 G>A, or 1138 G>C i.e. glycine to cytosine, which is relatively rare. These substitutions are readily detected by the gain of a SfcI or MspI site respectively.

Prenatal diagnosis is possible by serial ultrasonography for limb measurements, fetal X-rays after 20 weeks (should be avoided unless absolutely necessary) or by mutation testing using fetal DNA.

So far we have screened 39 subjects for both G>A and G>C mutations. Only four families had one parent with achondroplasia. Out of 39 cases, 25 (64.1%) had G>A transition and none was found having G>C transition. Five of those patients, in which neither of these two mutations were present, were screened for another mutation, G>T transversion at the first position of codon 375, leading to substitution of glycine to cysteine (G375C). However, none of these 5 subjects showed G>T mutation. Prenatal diagnosis (using chorionic villi samples) was offered to three families; in all cases the fetus was normal.

Prenatal Diagnosis in Fragile X Syndrome

Madhumita Roy Chowdhury, Madhulika Kabra, Deepti Sharma*, Deepika Singh*, Anjali Dabral*, BK Thelma*, Deepka Deka[#], Alka Kriplani[#], Veena Kalra

Genetic Unit, Department of Pediatrics, All India Institute of Medical Sciences, New Delhi, * Department

of Genetics, University of Delhi, South Campus, New Delhi. # Department of Obstetrics and Gynecology, All India Institute of Medical Sciences, New Delhi;

Fragile X syndrome is the most common cause of inherited X-linked mental retardation. It is due to a mutation in a gene on X-chromosome leading to hyperexpansion of a trinucleotide repeat sequence. The two most common fragile sites with clinical significance are FRAXA at Xq27.3 comprising CGG repeats and a more distal FRAXE associated with amplification of a GCC repeat, located at Xq28. The frequency of occurrence of Fragile X Syndrome is estimated to be 1/4000 male births.

It is a semi-dominantly inherited disorder with reduced penetrance. Males as well as females can be affected and in addition, both males and females can be unaffected carriers.

In our present study, we used a PCR based technique, which is a simple and rapid method of initial screening of samples and only samples tested positive with the above method were screened by southern blotting for confirmation.

Prenatal diagnosis was offered to six couples with positive family history and all the mothers were pre-mutation carriers. DNA from chorionic villus samples were screened for simultaneous amplification of the triplet repeat sequences at the FRAX loci, and then southern blot hybridization was carried out. Out of the six cases, 4 were females and 2 were found having expansion at FRAXA site and the remaining 2 fetuses were male and were diagnosed as normal.

Early Diagnosis of Autosomal Recessive Polycystic Kidney Disease- A Case Report

Renu Singh*, Vinita Das *, Manisha **Mandakini Pradhan **

Department of Obstetrics & Gynecology, King George's Medical University, Lucknow *, Department of Medical Genetics, SGPGIMS, Lucknow **

Autosomal recessive polycystic kidney disease (ARPKD) is a very rare entity (1 in 20,000 live births) and mostly is not compatible with life. It follows an autosomal recessive pattern of inheritance with siblings of either sex having a 25% chance of developing disease while the parents are unaffected. Here we report a nonconsanguineous couple referred at 24 weeks of gestation with enlarged kidneys on ultrasonography with history of intrauterine death at 28 weeks of gestation, decreased liquor in previous pregnancy. The fetus displayed clinical signs of Potter sequence. There was no history of polycystic kidney disease in the family. The couple had a normal renal ultrasound. High resolution ultrasonography revealed bilaterally enlarged echogenic kidneys with severe oligohydramnios in the present pregnancy. Autopsy and histopathological examination of kidneys and liver after termination of pregnancy confirmed the diagnosis of ARPKD. Termination of a further pregnancy at 18 weeks of gestation was undertaken after sonographic detection of large echogenic kidneys. Typical pathological changes were evident in the kidneys. Targeted ultrasonography of "at-risk" families helps in early detection of the malformation.

Prenatal Diagnosis of Duchenne Muscular Dystrophy Using Multiplex Polymerase Chain Reaction

Monica Tiwari, Vijaya R, Manjula Maheshwari, Sheffali Gulati, M.C. Sharma*, Chitra Sarkar*, Madhulika Kabra, Deepika Deka***, Alka Kriplani*** Sunita Mittal***, Veena Kalra****

Genetics Unit Department of Pediatrics, Division of Neurology Department of Pediatrics**, Department of Obstetrics & Gynecology***& Department of Pathology * All India Institute of Medical Sciences New Delhi

Duchenne Muscular Dystrophy (DMD) and Becker's Muscular dystrophy (BMD) are X-linked recessive disorders which affect around 1 in 3500 males. The DMD patients die in late teens or early twenties mostly due to involvement of heart muscles. BMD is a relatively milder form of the disease. There is no known cure of D/BMD. The only way is to prevent the birth of affected children by counseling and prenatal diagnosis.

DMD/BMD gene is located on the short arm of X chromosome and is a large gene. About 60-70% of affected individuals has one or more deletion(s) in the gene. The remaining mutations are mostly point mutations.

Methods: In the last seven years we analyzed a total number of 510 affected children and 115 Chorionic Villi samples in our molecular biology laboratory. All the samples were analyzed using multiplex PCR for detecting single or multiple deletions in the DMD gene. CA repeat analysis was done for the families in which deletion (s) could not be identified.