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Abstracts - Posters (Posters from the Delegates)

Ultrasound Diagnosis of Congenital Defects

R. A. Adhia, Z. M. Patel, S. Babu and A. K. D'Souza
Genetic Research Center, National Institute for Research in Reproductive Health, J.M. Street, Parel, Mumbai 400 012, Maharashtra, India
E-mail: zmpatel@hotmail.com

The objective was to study the role of ultrasound in the diagnosis of congenital malformations and chromosomal defects. A total of 2,000 pregnant women attended the clinic. All women had a maternal serum alpha fetoprotein and detailed ultrasonography at 16 weeks. Of the 2,000 cases scanned, 500 were "at risk" for neural tube defects, GI tract anomalies and kidney defects. Of these, 56 anomalies were detected. The karyotypic anomalies in amniotic fluid and fetal blood sampling were seen more frequently in cases with two or more abnormalities like cystic hygroma, hydrocephalus with diaphragmatic hernia and cardiac defects. In conclusion, fetal evaluation by ultrasound is an essential aspect in genetic counselling. Detecting of cytogenetic anomalies helps in further management of high risk pregnancies.

Study of the Extent of Awareness of Thalassemia within People

Maulik Bakshi and Dhiraj Mehta
Institute Jivraj Mehta hreasech Smarak Jivraj Park, Ahmedabad, Gujarat, India

The study attempted to assess awareness of disease of Thalassemia in important sectors (1) University students (2) Teachers and Professors of educational Institute (3) Medical staff of government hospitals (4) Thalassemia minors. The target groups were interviewed for their awareness about the disease in following sections with a set Performa of questions (A) General information—What is the disease? (B) Specific information - Nature and consequences (C) Counseling awareness —Premarriage and Prenatal counseling
The awareness extent was graded as (I) *Good*-If all three sections of inquiry satisfactory (II) *Fair*-If 1 and 2 sections satisfactory (III) *Poor*-All three sections unsatisfactory. All four groups were interviewed during Thalassemia minor testing project for 4500 university students funded by Rotary International under Thalassemia Awareness project of Rotary club of Ahmedabad, Metro Dist 3050. It was observed that (A) Out of 4500 students screened and interviewed , 0.4 %students had good , 0.8% had fair and 98.8 % students had poor awareness about the disease (B) Out of 54 teaching staff, 5.5 % staff had good,7.4 % had fair and 87 % had poor awareness about the disease (C) Out of 38 graduate {MBBS}Doctors in government hospitals only 5.5 percent had good, 13.15 had fair and 81% had poor awareness about the disease (D) Amongst 91 Thalassemia minors detected, only 1.82 % good ,7.69 had fair and 91.2 % had poor awareness about disease

Pre Natal Diagnostic Techniques (Regulation and Prevention of Misuse) Act 1994-Salient Legal Features

D. N. Bhardwaj[1], S. Lalwani[2], R. Rautji[3] and T. D. Dogra[4]
1. *Addl Professor,* 2. *Senior Resident,* 3. *Junior Resident,* 4. *Professor and Head*
Department of Forensic Medicine and Toxicology, AIIMS, New Delhi, India
E-mail: sanjulalwani2001@yahoo.com

Perturbed by the discrimination shown to female child and disturbed ratio of female to male in 1991

census in various states of Northern India due to female feticide, Govt. of India passed an act in parliament in 1994 to check and ban the discrimination of sex of child. It has enhanced the penalties on erring doctors/nursing homes carrying out such tests for MTP. Some Salient features pertaining to legal aspects of the act have been discussed in the poster

Prenatal Diagnosis of Merosin Deficiency Congenital Muscular Dystrophy

Sunita Bijarnia and I.C.Verma
Department of Genetic Medicine, Sir Ganga Ram Hospital, New Delhi 110 060, India
E-mail: dr_icverma@yahoo.com

Congenital Muscular Dystrophies (CMD) are genetically determined with a progressive clinical course. They are autosomal recessive and rare disorders. CMDs are characterised by a very early onset in first few months of life with generalized hypotonia, severe motor developmental delay, joint contractures and associated brain abnormalities. They could be merosin negative or merosin positive. The merosin negative is more severe and common.
We present here three cases of merosin deficient congenital muscular dystrophy (MDCMD) who were proven by special immunohistochemical staining of fresh frozen muscle biopsy specimen by monoclonal antibodies to merosin which is Laminin alpha 2 chain of muscle glycoprotein. In all three subjects, there was no staining with merosin antibodies as compared to a control muscle, the muscle specimen however stained well with control spectrin stain. In one case the diagnosis was confirmed by molecular linkage analysis. Based on this diagnosis, the three families were counselled regarding their risk of recurrence and the possibility of prenatal diagnosis by staining of chorionic villus sample, and /or molecular studies employing linkage in each pregnancy at 11-12 weeks of gestation. The Chorionic villus sample obtained was freshly frozen and stained with merosin antibody. All the three samples showed good staining with merosin thereby implying that the fetus was not likely to suffer from MDCMD. The diagnosis of normality was confirmed after delivery of the baby.
In conclusion, although the CMDs are rare diseases, the diagnosis of these disorders is possible by molecular analysis and accurate diagnosis in the affected child is essential prior to prenatal diagnosis.

FISH in Prenatal Diagnosis

Suresh Dhumal
Department of Assisted Reproduction and Genetics, Jaslok Hospital and Research Centre, Dr. G. Deshmukh Marg, Mumbai 400 026, Maharashtra, India
E-mail: arundhatia@yahoo.com

Fluorescence in situ hybridization (FISH) has recently been accepted as a new technique for genetic diagnostics. In our laboratory, it is used mainly to detect common aneuploidies in prenatal and postnatal samples for high-risk cases. We also use the technique on products of conception and bone marrow/leukemic blood in haematological malignancies. In prenatal diagnosis, the most common reason for referral is a high risk on Triple test. Hence FISH is mainly carried out on uncultured amniocytes, after pretreatment with trypsin and pepsin. Pretreatment is not required on CVS and cord blood samples. The reports are usually given in 2 days. This spares the expectant couple many days of anxiety awaiting a karyotype report. We simultaneously set up cultures for karyotyping to rule out other chromosomal aberrations whenever it is affordable by the patients. The probes used in our laboratory are commercially available, FDA approved and directly labelled (Aneuvysion –Vysis). Analysis is done using the Zeiss Axioskop 2 microscope with appropriate filters, a HBO 100 mercury lamp and Metasystems software. Coloured images are given in the report. We accept samples by courier from rural areas as well, where the patients can only afford to rule out Trisomy 21 by FISH in high-risk cases. Our experience with FISH on over 150 samples for prenatal diagnosis alone during the past 2 years is presented. Our laboratory is the first in India to offer FISH as a routine genetic

diagnostic test for prenatal diagnosis, PGD, products of conception and postnatal diagnosis in conditions ranging from infertility to mental subnormality and leukemia.

Interstitial Deletion of 18Q and Inversion 9QH: A Case Report

Vaishali Hydrabadi, Devendra Jhala, G. Avani, D. Chandel, Hyacinth Highland, M. V.Rao and G. J. Banke
Zoology Department, Gujarat University, Ahmedabad 380 00, Gujarat, India
E-mail: zooldeptgu@satyam.net.in

Deletions of chromosome 18q are among the most common segmental anuesomies compatible with life. Collectively, abnormalities of chromosome 18 form a major group of chromosomally abnormal children, next only to trisomy21 and fragile X syndrome. Individuals with monosomy 18q have variable regions deleted. Majority of them appear to have terminal deletions diagnosed using standard cytogenetic banding techniques. Interstitial deletions of varying lengths have also been reported, many of which were detected only after FISH and molecular analysis. We report here a case with a relatively large de novo interstitial deletion of chromosome 18q and an inversion 9qh. Inversion of the heterochromatic region of 9q is considered a normal cytogenetic variant. In the present case too, the inv(9qh), inherited from the phenotypically normal father appears a normal variant seemingly unrelated to the de novo monosomic condition of the patient.

Screening for Inborn Errors of Metabolism in Critically Ill Newborn

Anil B. Jalan[1] and Jayant Rewatkar[2]
1. *Chief Scientific Research Officer* (Bio-chemical Genetics)
2. *Neonatologist*
Navi Mumbai Institute of Research in Mental and Neurological Handicap [NIRMAN], 203, Nirman Vyapar Kendra, Sector 17, Vashi Navi Mumbai 400 705, Maharashtra, India

We sought to determine the incidence of various genetic and metabolic disorders in critically ill newborns in NICU setup and to determine the feasibility of comprehensive newborn screening for early identification of these disorders. Total 286 critically ill newborns admitted over last 4.5 yrs, in NICUs were studied in detail for genetic and metabolic disorders. The commonest IEM detected in our patients were Galactosemia (18), Biotinidase deficiency (8), Partial Biotinidase deficiency (24), MSUD (8), Tyrosinemia (1), Phenylketonuria (0), Homocysteinuria (1), CAH (4), Hypothyrodism (1), Propionic Acidemia (4), Methylmalonic Acidemia (2), Iso Valeric Acidemia (2), Fructose 1, 6-Diphosphatase Def. (1), Ketothiolase Deficiency (1), CPT 1 Def. (1), CPT II Def. (4), MCAD Def (1), LCHAD Def. (1), Severe Carnitine Def. (9), HMG Co A Lyase Def. (1), Respiratory Chain Defect (8), GA Type II (5), Cystic Fibrosis (3), G 6 PD Def. (2). Comprehensive metabolic screening for 30 + disorders will detect almost 68.13 % of all IEMs in these babies. We hereby strongly recommend comprehensive screening in all NICU babies.

Urinary Uric Acid and Creatinine Ratio as Screening Test for Possible GAMT Deficiency

Anil B. Jalan[1], V.S. Patkar[2] and Prerna Desai[3]
1. *Chief Scientific Research Officer* (Biochemical Genetics)
2. *Pathologist, MD DPB*
3. *Biochemistry* (M.Sc., Student of Mumbai University)

Navi Mumbai institute of Research in Mental and Neurological Handicap, NIRMAN, 203, Nirman Vyapar Kendra, Sector 17, Vashi Navi Mumbai 400 705, Maharashtra, India

We decided to determine normal urinary Uric acid / Creatinine ratios in mentally retarded children, who had normal Serum Uric acid levels. Once we develop the normal ranges for Indian children, this ratio can be used as a screening test for possible GAMT (Guanidinoacetate methyltransferase) deficiency. We screened 20 mentally retarded children with or without convulsions, above 1 year of age, with normal Serum Uric Acid. For this study we collected a morning urine sample and were analyzed for Urinary Uric Acid and Creatinine and the results were converted into mMol / L by standard conversion factor. Total 20 children studied 13 were males and 7 females. The mean and SD of Uric acid / Creat. Ratio (mol / mol) for males was 0.634 ± 0.268 and for Females was 0.612 ± 0.228. Any child with neurological involvement and Urinary Uric Acid / Creatinine ratio of > 0.6 mol / mol should be evaluated for possible GAMT deficiency.

Etiological Factors in Children with Autism

Anil B. Jalan[1] and Farzana Mulla[2]
1. *Chief Scientific Research Officer* (Bio-chemical Genetics)
2. *Clinical Psychologist* (Ph.D. Student of Neuro-psychology)
Navi Mumbai institute of Research in Mental and Neurological Handicap, NIRMAN, 203, Nirman Vyapar Kendra, Sector 17, Vashi Navi Mumbai 400 705, Maharashtra, India

We decided to study children with Autism, who attended our clinic from Jan. 2002 till July 31st 2003. The main aim was to determine inborn errors of metabolism or Fragile X syndrome as causative factors for Autism. We selected 34 children with Autism who attended our genetic clinic. Detailed metabolic investigations, Antigliadin Antibodies Ig G, Ig A and Tissue trans-glutaminase IgA were performed in all. Fragile X Chromosome was analyzed in 3 males by PCR technique and one female for Rett's syndrome. We found CMV infection (2/34). Gluten sensitivity (6/34), Partial Biotinidase deficiency (5/34), Biotinidase Def. (1/34) Phenylketonuria (1/34), Histidinemia (1/34), Homocysteinemia (1/34), Hyperleucinemia (1/34), Hypervalinemia (1/34), Argininemia (3/34), Respiratory Chain Defect (2/34), Succinyl Semialdehyde dehydrogenase def. (1/34), Fragile X Syndrome (1/34) and Rett's Syndrome (1/34), Incontinentia Pigmentii (1/34). 4 patients without etiological factor were labeled as probably Idiopathic. We could identify some etiological factor in 30 out of 34 children. We strongly recommend that all children with Autism should receive these basic investigations to rule out Gluten Sensitivity and Inborn Errors of Metabolism.

Gluten Sensitivity Screening in Children with Down's Syndrome

Anil B. Jalan[1], Judith Vaz[2] and Chandralata Sharma[3]
1. *Chief Scientific Research Officer* (Bio-chemical Genetics)
2. *Chief Neuro-developmental Physiotherapist*
3. *Speech Therapist and Audiologist*
Navi Mumbai institute of Research in Mental and Neurological Handicap, NIRMAN, 203, Nirman Vyapar Kendra, Sector 17, Vashi Navi Mumbai 400 705, Maharashtra, India

To estimate incidence of Gluten sensitivity in children with Down's syndrome. We screened 14 children with Downs's syndrome attending our genetic clinic. Gluten sensitivity was tested by Antigliadin Antibodies Ig G, Ig A, & Tissue Trans-glutaminase IgA. 7 out of 13 children (53.84 %) with Down's syndrome had all three titers positive. Since parents refused Endoscopy and biopsy for confirmation of coeliac disease, we started with GFD (Gluten Free Diet) in all 7 children with positive titers. 5 out of 7 children accepted and complied with GFD and were followed-up for 1 year. They were observed for development of milestones. There is definitely a higher incidence of Gluten sensitivity in the children

with Down's syndrome (> 50 %). It is our personal experience that Down syndrome children who comply with GFD show much faster gain in milestones and speech language. We therefore strongly recommend Gluten sensitivity screening in all children with Down's syndrome.

Tandem Mass Spectrometric Analysis for Aminoacids, Organic Acids and Fatty Acid Disorders in Critically Ill Newborns from Indian NICU

Anil B. Jalan[1] and Adolf Muehl[2]
1. *Chief Scientific Research Officer* (Bio-chemical Genetics)
NIRMAN, Navi Mumbai Institute of Research in Mental and Neurological Disorder, 203, Nirman Vyapar Kendra, Sector 17, Vashi, Navi Mumbai 400 705, Maharashtra, India
2. AKH Kinderklinik, University of Vienna, Austria.

There is no Indian data available for cutoffs, especially in critically ill newborns. It is estimated that 5 % of NICU babies will show abnormal markers (1). A prospective study was done over a period of last 2 years. 60 Critically ill newborns 46 males and 14 female were selected with age group ranging from 0-90 days (mean 18.18 days). All the patients were tested in duplicate. The parameters tested were Total Carnitine, Free Carnitine, Acyl Carnitines (C2-C18) and 10 different Aminoacids. For the purpose of calculation of normal ranges 6 abnormal patients are not considered and the mean, SD and Cutoffs (Mean + 3 SD) are calculated from the data of 54 normal patients. Cut off is taken as Mean + 3 SD as it corresponds to 99th centile and is the minimum cut off acceptable. Of these 60 babies, 6 had Inborn Error of Metabolism (one each of MMA, GA Type II, Hyperleucinemia, UCD, Hypermethioninemia and MSUD).

Clinical Spectrum of Glycine Encephalopathy in Indian Children

Anil B. Jalan[1] and Nandan Yardi [2]
1. *Chief Scientific Research Officer* (Bio-chemical Genetics)
2. *Paediatric Neurologist and Epileptologist*
Navi Mumbai Institute of Research in Mental and Neurological Handicap, 203, Nirman Vyapar Kendra, Sector 17, Vashi, Navi Mumbai 400 705, Maharashtra, India

Our aim was to determine clinical spectrum and outcome of NKH in Indian children. We identified 8 children with NKH in last 3 years**.** In all these 8 patients the Organic acidemia was ruled out by GC-MS or MS-MS or both. EEG was available in 7 children. CT scan or MRI was available in 6 patients. Diagnosis of NKH was based on the ratio of CSF / plasma Glycine > 0.08 with absence of Organic acidemia. Out of 8 children with NKH, 5 had neonatal onset of which 2 expired, 1 developed severe mental retardation, and two recovered completely (probably transient variety), 3 Children had late onset disease (after 3 months), of these none expired and all 3 had mental retardation. NKH is fairly common in India and outcome is unsatisfactory**.** Only 2 babies with NKH showed good clinical improvement (probably transient). We found that Lactate > 4.5 mMol/L and presence of hiccups in a critically ill child with NKH are bad prognostic signs.

Familial Supernumerary Marker Chromosome: Candidate for Prenatal Assessment?

Purvi M. Kakadia, Pina J. Trivedi, Manisha Brahmbhatt, Shwetal M. Rawal, Samarth S. Bhatt, Shakuntala V. Shah[1], Bhavna J Dave[2] and Sonal R. Bakshi
Cell Biology Division, Department of Cancer Biology, Division of Research,

1. Department of Surgical Oncology, The Gujarat Cancer Society, The Gujarat Cancer and Research Institute, NCH Campus, Ahmedabad 380016, Gujarat, India
2. Munroe Meyer Institute for Genetics and Rehabilitation, University of Nebraska Medical Centre, 600 South 42nd Street, Omaha NE 68198-5440, USA

Supernumerary marker chromosomes (SMC) are small extra chromosomes that are unidentifiable by cytogenetic banding techniques alone due to their small size and lack of distinct banding pattern. The incidence of marker chromosomes ranges between 0.4/1000 and 1.5/1000 in different studies. The precise characterization of SMC is important to know their phenotypic effect.
Here we report a rare case of familial SMC in a 50 years old female with tongue cancer. The constitutional cytogenetic analysis from blood culture revealed a mosaic 46,XX/47,XX,+mar.ish der(21)(q11…??)(80/100). Family study was undertaken, 11 members have been studied out of 18 members' family spanning three generations for karyotyping. The marker chromosome was observed in four, i.e. two sons (age 22 & 28 years), a daughter (age 29 years), and a grand son (age 6 years). All of them have no abnormal phenotypes like dysmorphic features, bad obstetric history, or mental retardation.
The SMC was smaller than a G-group chromosome. Conventional banding techniques viz. QM; GTG, CBG and Ag-NOR were non-informative for identification. Multiplex fluorescence in situ hybridization (M-FISH) was performed with Cytovision Probe workstation (Applied Imaging), which revealed the SMC origin to be chromosome 21. The WCP 21(VYSIS Inc.) FISH confirmed the same in other individuals carrying SMC.
To the best of our knowledge, this is the first report of familial SMC derived from HC21 in a patient with adult-onset tongue cancer and normal family members. The SMC carriers will be followed up, the youngest being two years old. The family has also been requested to check for prenatal assessment for the presence of SMC in future for academic interest. Further studies are required to know the significance of this observation.

Cytogenetics Study in Recurrent Spontaneous Aborters

V. Lakshmi Kalpana and M. Satyanarayana
Department of Human Genetics, Andhra University, Vishakhapatnam 530 003, Andhra Pradesh, India
Email: anuv2k@yahoo.com

Reports of females with X chromosomal mosaicism and pregnancy losses are numerous. To find out the presence of the aneuploidy chromosomal analysis was performed in a series of 30 women with repeated spontaneous abortions and their husbands.
Out of 60 individuals a 28 years female who was experienced four spontaneous abortions was detected to be a chromosomal mosaic 45, X / 46, XX / 47, XXX with corresponding sex chromatin picture –ve/+ve/++ve. She had normal phenotype and abortions that occurred in the second/third months of pregnancy. Her husband has normal male chromosomal complement (46, XY).

FISH (Fluorescence *in Situ* Hybridization) in Products of Conception

Mahadev Kawle
Depatment of Assisted Reproduction and Genetics, Jaslok Hospital and Research Centre, 15, Dr. G. Deshmukh Marg, Mumbai 400 026, Maharashtra, India.
E-mail: arundhatia@yahoo.com

Fluorescence *in situ* hybridization (FISH) is a molecular cytogenetic technique that has multifaceted uses in routine genetic diagnostics, mainly for detection of aneuploidies. FISH is a rapid and reliable technique where the reporting time is about 2-3 days as against 2 weeks by culture techniques. FISH

is possible on many tissues, direct or cultured, from products of conception (POC) like chorionic villi, skin and placenta. Karyotyping is not always successful in POC due to contamination, degeneration of the tissue or insufficient villi. Even in such cases, a report of the common aneuploidies is still possible on interphase cells from tissue that has already been processed and fixed for karyotyping by any other laboratory. One or two strands of villi also give sufficient interphase cells for analysis. In our laboratory, if karyotyping from POC fails, we give an alternate FISH report at no extra cost.. We have been able to detect cases of Turner syndrome, Klinefelter syndrome, trisomies 13, 18, 21 and mosaicism very easily by the FISH technique. We use a Zeiss Axioskop microscope with appropriate filters and the Metasystems software to generate coloured images for the report. The probes we use are FDA approved (Vysis Aneuvysion) and the hybridization protocol we follow is the rapid procedure described by the manufacturers. Our data of FISH on 82 samples of products of conception analyzed over a two and a half year period at Jaslok Hospital and Research Centre will be presented. Aneuploidy was detected in 20 cases, besides many cases of mosaicism.

Prenatal Diagnosis of Phenylketonuria

S. Kohli, R. Saxena and I. C. Verma
Department of Genetic Medicine, Sir Ganga Ram Hospital, New Delhi 110 060, India
E-mail: kohli_sudha@yahoo.com

Phenylketonuria (PKU) is a common inborn error of metabolism, which if untreated leads to mental retardation. It is caused by deficiency of phenylalanine hydroxylase (PAH) activity, which is expressed only in the liver. Because of the cost of dietary treatment, parents in India desire a prenatal diagnosis (PND). However PND cannot be done by measuring phenylalanine level in the fetal blood, while measurement of phenylalanine hydroxylase (PAH) enzyme activity requires biopsy of fetal liver. The right approach for PND of PKU therefore is by molecular methods. We report a couple who were married consanguineously and had one daughter with severe mental retardation, light colored skin and hair. The serum phenylalanine was 800mg/dl and the urine had increased phenylpyruvic acid. They desired prenatal diagnosis in the current pregnancy. This was done by linkage analysis of markers linked to the PAH gene chromosome 12q24.1. Testing for short tandem repeats (STRs) in intron 3, VNTR and Xmn1 RFLP, revealed that the fetus had inherited a normal chromosome from the mother and an affected chromosome from the father. Carrier status for PKU was hence diagnosed in the fetus. The diagnosis of being unaffected was confirmed by tests on the newborn.

A Study of Semen Analysis, Karyotyping and Y Chromosome Microdeletions in Infertile Indian Males

Dattatray Naik
Department of Assisted Reproduction and Genetics, Jaslok Hospital and Research Centre, Dr. G. Deshmukh Marg, Mumbai 400026, Maharashtra, India.
E-mail: naikdatta72@rediff.com

Male partners of infertile couples are frequently observed with abnormal semen parameters. Many cases also show genetic basis. In order to determine the cause of male infertility, clinical investigations should include semen analysis, karyotyping and DNA analysis to rule out Y chromosome microdeletions. At our clinic we undertook a study of male partners of infertile couples to find out the correlation between abnormal sperm parameters, karyotypes and Y chromosome microdeletions. Another aim was to determine the frequency and common loci of Y chromosome microdeletions among infertile men in India. The male partners were tested by semen analysis. From these, 93 patients with abnormal semen parameters like oligo/ astheno/ terato/ azoospermia or combinations of these were selected.

They were tested for routine karyotyping using a Zeiss Axioskop 2 microscope along with Metasystems software. Multiplex PCR analysis for 18 loci on the Y chromosome was carried out using commercial kits (Promega Version 1.1). Chromosomal abnormalities/ variants were detected in 3/93 patients. Two had balanced translocations and 1 was a variant. Y chromosome microdeletions were observed in 12/93 patients (12.90%) including 8/25 males with azoospermia (32%), 1/8 with asthenoteratozoospermia (12.5%) and 3/49 with oligoasthenoteratozoospermia (6.12%). All loci of the DAZ gene were deleted along with DYS237 and DYS236 from AZFd in 4/25 azoospermic males studied. DYS240 (11/12) and DYS219 (6/12) were the most commonly deleted loci. In an azoospermic male with a karyotype of 45,X,t(Y;13)(q11.2;q11),-der13 confirmed by FISH analysis using the Vysis Aneuvysion probe kit, Y chromosome microdeletions were detected in 16/18 loci. In another case where all the sperms had pinheads, the karyotype and Y microdeletion analysis were normal. Thus, a routine semen analysis, karyotyping to rule out structural and numerical chromosome abnormalities and screening for Y chromosome microdeletions help in the management of couples with male factor infertility.

HPLC Diagnosis of β-Thalassemia Major on Fetal Blood Samples

I. Panigrahi , M. Kabra, D. Deka and R. Saxena
Department of Hematology, All India Insititute of Medical Sciences, Ansari Nagar, Delhi 110 029, India
E-mail: inupan@yahoo.com

HPLC (High-Performance Liquid Chromatography) is an accepted method for the diagnosis of hemoglobinopathies. The method was tested in this study for the diagnosis of thalassemia major in fetal blood samples. Prenatal diagnosis was performed for couples with previous thalassemia major child. The affected children had one identified and one unidentified mutation on DNA analysis. Cord blood was obtained at 18 – 23 weeks of gestation and HbA, HbF and HbA2 fraction were determined on hemolysates. Of four samples tested, one fetus was affected and three were unaffected. The affected fetus had HbA level of 0.2 %. Cord-blood HPLC may be acceptable alternative where a) DNA diagnostic facilities are not available b) mutation is unidentified despite both parents being carriers on screening tests; c) at-risk mother comes late in pregnancy for diagnosis tests. Further studies in Indian population are warranted.

Ascertainment of Chromosome 22Q11.2 Micro Deletion in Congenital Heart Defects: FISH Study Of 90 Cases

Z.M. Patel, H.M. Gawde, A.K. D'Souza and S. Babu
Genetic Research Center, National Institute for Research in Reproductive Health, J.M. Street, Parel, Mumbai 400 012, Maharashtra, India
E-mail: zmpatel@hotmail.com

Micro deletion of chromosome 22q11.2 is associated with a highly variable phenotype that includes DiGeorge, Velocardiofacial and conotruncal anomaly face syndrome as well as apparently isolated congenital heart defects. It has been estimated that deletion at 22q11.2 occur in 1:4000 newborns and majority of deleted patients share a common 3Mb hemizygous deletion of 22q11.2. This study reports FISH results of 90 cases presenting clinically with a spectrum covering isolated congenital heart defects, Tetralogy of Fallot (TOF), Ventricular Septal Defect (VSD), Truncus Arteriosus (TA) and Atrial Septal Defect (ASD). FISH technique applied to blood lymphocyte metaphase spreads and interphase nuclei using dual colour DNA probe specific 22q11.2 and to the band 22q13 as a control region (VYSIS) revealed a normal status of chromosome 22 in all the patients except for one, where the mother was confirmed to the mosaic for microdeletion on 22q11.2 (10 % of her cells showed hemizygous

deletion). The deletion on 22q11.2 was not detected by classical cytogenetic technique in this case. This shows low frequency of deletion (1.2%) in our study as compared to other genes.
Parents with 22q11.2 deletion have a 50% risk of transmitting the deletion to their offspring.
We present prenatal diagnosis of Ch 22q using FISH technique.

Fertility in a Male with Trisomy 21

M.Pradhan, Faizal Khan, J. Patil, Shubha R. Phadke and S. Agarwal
Department of Genetics, S.G.P.G. Institute of Medical Sciences, Lucknow 226 014, Uttar Pradesh, India

Down syndrome(trisomy 21) is the most common chromosomal abnormalities, occuring in about 1 in 700 live births. It is the only autosomal trisomy with post-pubertal survival. Hence the possibility of fertility in these patients is often raised. Many pregnancies have been reported in female with Down syndrome. However, so far only two males with Down syndrome have been reported to be fertile. Here, we report the serological, andrological data of a male with non mosaic Down syndrome who fathered a child. The paternity has been proven using DNA fingerprinting.

Molecular, Cytogenetic and FISH Analysis of a Tandem Translocation of Chromosome 21 t(21;21)(q22.3;q22.3) in a Mildly-affected Patient with Down Syndrome

Frenny Jayesh Sheth [1], Uppala Radhakrishna [2], Jean-Louis Blouin [2], Michel A Morris [2], Arnaud Gos [2], Jayesh J Sheth [1], Asha S Multani [3] and Stylianos E Antonarakis [2]
1. Genetics Centre *, 20/1 Bima Nagar Society, Satellite Road, Ahmedabad, Gujarat, India
2. Laboratory of Human Molecular Genetics, Department of Genetics and Microbiology, Geneva Medical School and Division of Medical Genetics, Cantonal Hospital, Geneva, Switzerland
3. Department of Cell Biology, University of Texas, Houston,Texas, U.S.A.

A five-year-old male was referred for cytogenetic analysis because of mild mental retardation and facial features typical of Down syndrome. He was the second child born to non-consanguineous parents; his older sister and younger brother were normal. Many of the typical features of Down syndrome were present in the proband, including neonatal hypotonia, macroglossia, heart defect, and flat nasal bridge. Hands and eyes are normal. Cytogenetic study of peripheral blood lymphocytes using GTG banding revealed the karyotype 46,XY,-21,+t(21;21)(q22.3;q22.3). Over 400 metaphases were analysed and no mosaicism was detected. No other family member, including the parents, had the chromosomal anomaly. Fluorescence *in situ* hybridisation (FISH) using a half YAC containing the telomere of chromosome 21q and the adjacent marker *D21S1575* revealed the deletion of both copies of this region on the translocated chromosome. Microsatellite analysis further delineated the breakpoint to between *CD18* and *D21S1446*, excluding the possibility of uniparental disomy, and demonstrated that the rearranged chromosome was of paternal origin.

Possible Interchromosomal Effect and Increased Risk of Trisomy

Frenny J.Sheth, Trupiti N. Patel, Meghna Kumar, Dominic Mao, Bhaomi Desai and Jayesh J.Sheth
Foundation for Research in Genetics and Endocrinology [Frige], Genetic Centre, 20/1, Bimanagar, Satellite, Ahmedabad 380 015, Gujarat, India
E-mail: jshethad1@sancharnet.in

The population risk for trisomy 21 is 1 in 700 births but some couples are at a much higher risk owing to parental translocation or mosaicism. We present a case where amniocentesis was carried out at 16

weeks of pregnancy having a family history of one Mongol child. He was not investigated postnatally for genetic studies. Following this, mother had two consecutive first trimester abortions. Genetic amniocentesis during forth pregnancy showed Robertsonian translocation involving chromosome 13 and 14 with normal sex chromosomes. Looking to the family history, all the members of the family were cytogenetically investigated to rule out the inheritance of Robertsonian translocation, which confirmed to be of paternal origin. First child born to the non-consanguineous parent showed double aneuploidy having Robertsonian translocation involving #13 and #14 along with free trisomy 21.
This shows that such couples carries 1% risk of having chromosomally imbalance offspring for carriers of balanced structural rearrangement at amniocentesis. In addition, the risk increased by 2.5% including aneuploidy of other chromosome has been observed in present case.

Triple Marker Study During 2nd Trimester Followed by Cytogenetic Study

Jayesh Sheth, Frenny Sheth, Nrupesh Oza and Minesh Doshi
FRIGE, Genetics Centre, 20/1, Bimanagar, Satellite Road, Ahmedabad 380 015, Gujarat, India

Triple Marker Study was carried out in 1,296 women in the age range of 20 to 47 years. They were divided in two groups as those below 35 and above 35 yrs. Of 946 women in the age range of 20-35 yrs, 68 (7.19%) were screen positive for trisomy 21 and 20 (2.11%) women had raised AFP. 96 (27.43%) of 350 women were screen positive for trisomy 21 in the age range of 35 to 47 yrs and 12 (3.43%) had raised AFP. The overall false positive rate for Down syndrome screening was 12.65%. Of 164 women screened positive, 72 (43.9%) had opted for further confirmation by amnio culture study, one women had aborted and came for cytogenetic study while 92 women have decided either for termination or continued the pregnancy. Sixty of 72 (44%) women have shown normal amniotic fluid cytogenetic study while 11(15.41%) women have shown chromosomal abnormality. Eight of these have shown free trisomy 21, one with mosaic for Down syndrome, one with t(13;14); one with marker chromosome and one had shown polyploidy later on experienced pregnancy loss.
Out of 28 women with raised AFP (MoM >2.5), 3 (10.71%) had shown confirmed neural tube defects in the fetus while rest were normal. Though no follow-up was available in rest of the cases.
We conclude from this study that false positive rate for TMS is almost four times higher in elderly women as compared to the young women. The overall risk of cytogenetic abnormality in screen positive women is 15.41% of which two third women had confirmed trisomy 21 while one third women have shown structural, numerical anomalies which would have been missed if the decision of pregnancy continuation was made on normal nuchal fold thickness ignoring triple marker results. The overall acceptance rate for amnio was about 50% suggesting the need for proper genetic counseling by the physician ordering the test and the geneticist. This study also suggests that false elevation of AFP is not uncommon and decision should be made in such cases based on other findings like sonography.

MTHFR (C677T) Polymorphism and Homocysteine Metabolism in Mothers with Down Syndrome Child

Jayesh Sheth, Frenny Sheth, Minesh Doshi and Nrupesh Oza
Foundation For Research in Genetics and Endocrinology [FRIGE] Genetic Centre, 20/1, Bimanagar, Satellite, Ahmedabad 380 015, Gujarat, India

Down syndrome is the most common cause of mental retardation with live birth frequency of 1:700 to 1:1000. The study was carried out in 28 case mothers and 13 age matched control mothers. MTHFR gene polymorphism and biochemical markers such as Folate, cobalmin, homocysteine, cysteine, methionine and glutathione were carried out in all women. Seven (25 %) case mothers had shown

heterozygous (CT) genotype, while 6 (46%) control mothers had shown the similar pattern. Twenty (71 %) case mothers had wild type (CC) genotype as compared to 6 (46 %) control mothers, while homozygosity (TT) was observed in one mother in both the group. Plasma level of cobalmin (Vit B-12) (708.88 ± 202.07 pg/ml vs. 560.90 ± 246.35 in case and control mothers respectively) and folate (10.89 ± 4.28 vs. 10.27 ± 4.96 ng/ml) were identical in both the groups. Plasma homocysteine was significantly raised (8.82±2.4 mmol/L) in case mothers as compared to control mothers (6.49±1.83 mmol/L, P= 0.025). Plasma methionine (25.09±5.16 vs. 21.52±6.05 mmol/L, p=0.172) and glutathione (5.13± 1.31 vs. 4.6±0.99, p=0.486) were low in case mothers as compared to control mothers though no statistical significance was observed.

We can conclude from the present study that MTHFR polymorphism (C677T) genotype is likely to be commonly observed in Indian Gujarati women. The cause of hyperhomocysteinemia may be due to several mechanisms.

a. Deficient activity of enzyme betaine homocysteine methyltransferase.
b. Mutation in cystathione-Beta-synthase gene involved in homocysteine transsulphuration or
c. Nutritional factors and gene interactions.

Multicentre trials are needed to confirm or refute the observations

Prenatal Diagnosis/ PGD By Karyotyping/ FISH/ PCR

Lata Shewale
Reliance Life Sciences, Department of Assisted Reproduction and Genetics, Sir H. N. Hospital and Research Centre, Mumbai 400 004, Maharashtra, India
E-mail: prochi_madon@relbio.com

Pregnancy is a time of excitement and wonder, tempered by natural concerns about the well being of the developing fetus. But all fetuses are not normal at birth. To determine the health and condition of an unborn fetus, a variety of techniques are employed referred to as Prenatal Diagnosis (PND). Prenatal diagnosis is offered to couples who are at risk for pregnancy complications such as Advanced maternal age Family history of birth defects, Single gene disorders Abnormal ultrasound findings High risk on Triple test screening. There are a variety of non-invasive and invasive techniques available for prenatal diagnosis. Each of them can be applied only during specific time periods during the pregnancy for greatest utility. Over a period of 2 years at Reliance Life Sciences (RLS) we offer PND at all gestations to rule out chromosomal abnormalities by Karyotyping and FISH techniques. In addition we offer PND for single gene disorders such as Thalassemia, HbS and HbE by ARMS-PCR. We have also established preimplantation genetic diagnosis (PGD) of aneuploidies by the FISH technique. In PGD, embryos free of common aneuploidies are identified and transferred to the mother during the IVF cycle. Our experience on 75 cases over the past 2 years will be described.