



International Conference on Medical & Community Genetics

**Theme: Current issues in Diagnosis, Management,
Genetic Counselling & Population Diversity**

February 15th-17th, 2008

Abstract Book

Organized by

Department of Hematology

Postgraduate Institute of Medical Education & Research

Chandigarh - 160 012 (India)

PROGRAMME

Day One: 15th February, 2008

Registration	8.00 am onwards
Inauguration	9.00 am – 10.00 am
Tea Break	10.00 am – 10.30 am

Scientific Sessions

Session- I: 10.30 am - 11.45 am

Chairpersons: Prof. B. N. S. Walia and Prof. R. C. Sobti

10.30 am - 11.15 am: **Keynote Speaker:**

Prof. Alan Bittles (Perth, Australia)
The Brave New World of Medical Genetics

11.15 a.m.- 11.45 am Prof. I. C. Verma (New Delhi, India)
Challenges & opportunities in genetic disorders in India

Session- II: 11.45 am- 1.05 pm

Chairpersons: Dr Prof. Nancy Olivieri and Dr Meenu Singh

11.45 am- 12.15 pm Prof. Michael Patton (London, UK)
Is murder genetic?

1215 pm- 12.40 pm Dr Madhulika Kabra (New Delhi, India)
Challenges of Medical Ethics in genetic disorders

12.40 pm- 1.05 pm Prof. Shubha Phadke (Lucknow, India)
Genetic counselling

1.05 pm – 2.00 pm **Lunch and Poster Viewing**

Session- III: 2.15 p.m.- 3.30 pm

Chairpersons: Prof. Ajay Wanchu and Dr Ranjana Minz

2.00 pm- 2.25 pm Prof. N. K. Mehra (New Delhi, India)
Predictive cytokine polymorphic genes in organ & BM transplantation

2.25 am – 2.50 pm Dr Swapan Nath (Oklahoma,, USA)
Positional identification of a Lupus susceptibility gene

Chairpersons: Dr Vivekananda Jha and Dr Sunil Arora

2.50 pm – 3.15 pm Dr Rama Mittal (Lucknow, India)
Association study of pro/anti Inflammatory Cytokine genes
polymorphism with allograft rejection risk in renal Tx patients

3.15 pm – 3.40 pm Dr G. R. Chandak (Hyderabad, India)
Unraveling the etiopathogenesis of chronic pancreatitis In India

3.40 pm -4.00 pm **Tea Break**

Session- IV: 4.00 pm – 5.00 pm

Chairpersons: Prof. K. K. Kohli and Prof. Tapas Mukhopadhyay

4.00 p.m.- 4.30 pm Dr Ken McElreavey (Paris, France)
Genetics of the human Y chromosome

4.30 pm -5.00 pm Prof. Wei Wang (Beijing, China)
Population diversity and health in China

5.30 pm- 7.00 pm **Presentations and Evaluation of Posters**

Day Two: 16th February, 2008

Session- V: 9.00 am – 10.45 am

Chairpersons: Prof. Vanita Jain and Dr Amita Trehan
9.00 am – 9.30 am Dr Samuel S. Chong (Singapore)
Preimplantation genetic diagnosis: relevance to Paediatrics & Obstetrics

9.30 am – 9.55 am Dr Urvashi Trehan (USA)
Introducing new platforms for newborn screening of genetic diseases

9.55 am – 10.15 am Dr Usha Dave: (Mumbai, India)
Newborn screening for IEM disorders

10.15 am-10.30 am **Tea Break**

Session- VI: 11.00 am – 1.00 pm

Chairpersons: Prof. Neelam Varma and Prof. Balraj Mittal
10.30 am – 11.00 am Dr Radhakrishna Uppala (Geneva, Switzerland)
Molecular genetic analysis of congenital limb anomalies

11.00 am – 11.30 am Dr Anand Saggarr (London, UK)
Polycystic kidney diseases - from disease to gene and back

11.30 am – 11.50 am Dr Dhananjaya Saranath (Mumbai, India)
Clinical Implications of Molecular Genetic Diagnostics

11.50 am – 12.10 Prof. Madhu Khullar (Chandigarh, India)
Molecular Genetics of Non Syndromic Hearing Loss

Chairpersons: Prof. Akhtar Mahmood and Prof. Anuradha Chakrabarti
12.10 pm – 12.30 pm Dr Ken McElreavey (Paris, France)

Genetics of human sex determination and differentiation

12.30 pm – 12.50 pm Dr Jayesh Seth (Ahmedabad, India)

Lysosomal storage disorders in India: Present Scenario.
12.50 pm – 1.10 pm Prof. Rajendra Prasad (Chandigarh, India)
Molecular genetics of Wilson's disease

1.10 pm – 2.00 pm **Lunch and Poster Viewing**

Session- VIII: 2.00 pm – 3.40 pm

Chairpersons: Prof. R.K. Marwaha and Prof. B.S. Shah
2.00 pm – 2.25 pm Dr Kanjaksha Ghosh (Mumbai, India)
Genetics and therapy of Haemophilia in Developing Countries

2.25 pm – 2.50 pm Prof. Renu Saxena (New Delhi, India)

	Rare bleeding disorders
Chairpersons:	Prof. Harsh Mohan and Prof. MA Khan
2.50 pm – 3.15 pm	Prof. Subhash Varma (Chandigarh, India) Clinician's approach to prothrombotic disorders
3.15 pm – 3.40 pm	Dr Amar Dasgupta (Mumbai, India) Issues in the Diagnosis of Thrombophilic Disorders
3.40 pm - 4.00 pm	Tea Break
Session- IX:	4.00 pm – 5.30 pm
Chairpersons:	Prof. Deepak Kaul and Dr Radhika Srinivasan
4.00 pm – 4.15 pm	Dr Vijay P. S. Rawat (Munich, Germany) Role of Homeobox Gene Cdx2 in Human Leukemia.
4.15 pm – 4.30 pm	Dr A. Ghaffar (Lahore, Pakistan) Increased susceptibility of Galectin-3 knockout mice
4.30 pm – 4.45 pm	Dr M. Aslamkhan (Lahore, Pakistan) Cultural consanguinity in the Punjab, Pakistan
4.45 pm – 5.00 pm	Dr Salma Iqbal Naik (Lahore, Pakistan) Impact of consanguinity on the spread of thalassemia in Lahore, Pakistan
5.00 pm – 5.10 pm	Ms Aysha Azhar (Faisalabad, Pakistan) Molecular genetic analysis of three autosomal recessive skin disorders in Pakistan: ectodermal dysplasia, alopecia & Nail dysplasia
5.10 pm – 5.20 pm	Mr Shoab ur Rehman (Faisalabad, Pakistan) Molecular genetics of AR primary microcephaly (MCPH) in Pakistani families
5.30 pm – 6.30 pm	Thalassaemia Consensus Meeting

Day Three: 17th February, 2008

CME ON THE THALASSAEMIA SYNDROMES

Session- I:	9.00 am – 10.30 am
Chairpersons:	Prof. K. C. Das and Prof. G. Garewal
9.00 am – 9.40 am	Keynote Speaker Prof. Sir David Weatherall (Oxford, UK) The population genetics and dynamics of the haemoglobinopathies
9.40 am – 10.05 am	Dr Nishi Madan (New Delhi, India) Role of Red cell indices to screen for Beta Thalassemia Trait
10.05 am – 10.25 am	Dr. Anil Handoo (New Delhi, India) Screening of thalassemsias and hemoglobinopathies in the current era
10.25 am-10.45 am	Tea Break
Chairpersons:	Dr P. K. Gogai and Dr Jena
10.45 am – 11.05 am	Dr Dipika Mohanty Suitable method(s) for Beta thalassemia carrier detection in India– Experience from a multicentric large population study
11.05 am – 11.20 am	Dr R. S. Balgir (Bhuvaneshwar, Orissa)

11.20 am - 11.35 am Phenotypic Diversity of Congenital Sickle Cell Disorders
Dr B.P. Dash (Balasore, Orissa)
Spectrum and biology of sickle cell disorders in Orissa state, India

11.35 am - 11.55 am Dr Utpal Chaudhuri (Kolkata, India)
Hb E disease in India

Chairpersons: Dr Ajit Gorakshakhar and Dr Reena Das

11.55 am - 12.15 pm Dr Sarita Aggarwal (Lucknow, India)
Alpha thalassemia in modifying the phenotypes of beta thalassemia

12.15 am - 12.35 pm Dr Sharmila Chandra (Kolkata, India)
Alpha thalassemia in Eastern India

12.35 pm - 1.00 pm Dr Roshan Colah (Mumbai, India)
Invasive & non-invasive prenatal diagnosis of thalassemia syndromes:
Feasibility & acceptance by the community"

1.00 pm – 2.00 pm **Lunch**

Chairpersons: Prof. Subhash Varma and Prof. R. K. Marwaha

2.00 pm- 2.30 pm Dr Nancy Olivieri (Toronto, Canada)
Secondary iron overload and Iron Chelators in Thalassemia

2.30 pm- 3.00 pm Prof. Mammen Chandy: (Vellore, India)
Bone marrow transplantation: Vellore experience

Chairpersons: Prof. Neelam Marwaha and Dr K. Ghosh

3.00 pm- 3.30 pm Dr Rajat Kumar (New Delhi, India)
Cord blood storage for thalassaemia patients

3.30 pm - 4.00 pm Dr Punam Malik (Cincinnati, USA)
Progress in gene therapy for thalassaemia

4.00 pm - 4.30 pm **Tea Break**

4.30 pm – 5.45 pm Panel Discussion for Young Adult Thalassaemics

Coordinator: Ms Alka **Participants:** Mr Vipul, Mr Gagan and Mr Rishi

Chairpersons: **Sir Prof. David Weatherall and Prof. K. C. Das**

Panelists: Dr N. Olivieri, Dr G. Garewal, Dr R. K. Marwaha, Dr Punam Malik,
Dr K. Ghosh & Mrs Shobha Tuli

5.45 pm– 6.00 pm **Concluding Remarks**

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BP1

VEGF-1154G/A GENE POLYMORPHISMS IN METABOLIC SYNDROME IN NORTH INDIAN POPULATION: IMPACT ON CORONARY ARTERY DISEASE: A CASE-CONTROL STUDY

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Introduction: Vascular endothelial growth factor (VEGF), is a potent angiogenic factor and neovascularisation has been shown to be important in atherosclerotic plaque development. *VEGF* –2578 genotypes have shown a significant association with atherosclerosis.

Objectives:

1. To examine association between *VEGF* -1154 G/A gene polymorphisms and metabolic syndrome (MS) as well as coronary artery disease (CAD).
2. To evaluate the role of *VEGF* gene in CAD and MS.

Methods: 100 cases of MS as per NCEP-ATPIII criteria, 200 CAD-post angioplasty patients and 200 healthy controls were enrolled. Genomic DNA was isolated from whole blood using chloroform-phenol method. To check polymorphism of *VEGF*-1154G/A gene on MS and CAD, polymerase chain reaction - amplification refractory mutation system (PCR-ARMS) was carried out. Chi square test was done using SPSS 12.0 version to calculate the frequencies.

Results: Frequencies of A , AG and GG genotype were 51.5% , 47% and 1.5% in CAD, 43%, 31% and 26% in MS , 20% ,64% and 16% in controls. AA genotype was significantly higher in CAD ($OR=3.506$; $95\%CI=1.857-6.616$, $p=0.000$,) and MS ($OR=4.439$; $95\%CI=2.252-8.746$, $p=0.000$) as compared to controls. Frequency in males was significantly higher in CAD ($OR=2.936$; $95\%CI=1.729-4.985$, $p=0.000$).

Conclusions: *VEGF-1154G/A* gene is associated with susceptibility to CAD and MS. There might be a significantly higher impact in the development of MS as compared to CAD. The *AA* genotype may be a major risk factor in north Indians with MS and CAD. This study provides preliminary evidence that *VEGF-1154G/A* gene is associated with development of MS and CAD.

BP2

CYCLOOXYGENASE-2 GENE POLYMORPHISMS CONFER DIFFERENTIAL SUSCEPTIBILITY FOR *HELICOBACTER PYLORI* INDUCED GASTRITIS AND GASTRIC ATROPHY IN NORTHERN INDIANS

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Introduction: Cyclooxygenase (COX)-2 catalyzes conversion of arachidonic acid to prostaglandins which further mediates inflammation. COX-2 is upregulated in *Helicobacter pylori* induced gastritis, precancerous lesions and gastric cancer.

Objectives: We evaluated the association of functionally relevant polymorphisms of *COX-2* gene (-765 G>C and +8473 T>C) with gastritis and precancerous lesions.

Methods: After upper GI endoscopy, 130 rapid urease test positive patients with non-ulcer dyspepsia who also had positivity for *H. pylori* using modified Geimsa staining in gastric biopsy or serology for anti-CagA IgG were included. All patients and 260 asymptomatic controls were genotyped for *COX-2* variations using polymerase chain reaction followed by restriction fragment length polymorphism.

Results: Frequencies of *COX-2* -765 (GC+CC) genotypes, -765 C allele, +8473 CC genotype, +8473 C allele, and variant haplotypes (T-C, C-G and C-C) were higher in patients than controls which imparted high risk for gastritis (P=0.036, OR=1.82; P=0.007, OR=1.92; P=0.017, OR=1.80; P=0.017, OR=1.45; P=0.019, OR=2.40; P=0.023, OR=1.50 and P=0.012, OR=2.21 folds, respectively). In contrast, *COX-2* -765 C allele carriers imparted low risk for infiltration of lymphocytes (P=0.020, OR=0.35), plasma cells (P=0.016, OR=0.33) and development of gastric atrophy (P=0.019, OR=0.35).

Conclusions: Individuals with *COX-2* variant alleles, genotypes and haplotypes may be at high risk for gastritis development. In contrast, *COX-2* -765 C allele carriers are at low risk for gastric

atrophy. Study suggests that gastritis patients with *COX-2* variant alleles may be protected from progression towards gastric atrophy that may explain low incidence of gastric cancer in India.

BP3

MICROSOMAL EPOXIDE HYDROLASE POLYMORPHISMS (EPHX1): INFLUENCE OF SLOW (EXON 3, 113HIS) AND FAST ALLELE (EXON 4, 139ARG) ON SUSCEPTIBILITY OF SQUAMOUS CELL ESOPHAGEAL CANCER

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Introduction: Genetic polymorphisms in xenobiotic metabolizing enzymes may alter risk of various cancers.

Objectives: Present case-control study evaluated the influence of *EPHX1* genetic variations on squamous cell esophageal cancer (ESCC) susceptibility.

Methods: A total of 107 patients and 320 controls were genotyped for *EPHX1* polymorphic alleles by direct sequencing (exon 3, *Tyr113His*) or PCR-RFLP (exon 4, *His139Arg*).

Results: In patients, high frequency of exon 3 genotypes (*Tyr113His*, *His113His*) and 113His alleles was observed in comparison to controls which imposed two fold risk for SCC (OR 2.0, 95%CI=1.2-3.4, p=0.007; OR 2.3 95%CI=1.0-5.2, p=0.03 and OR 1.5, 95%CI=1.0-2.1, p=0.01). In contrast, individuals with exon 4 *139Arg* alleles were at low risk of cancer (OR 0.34, 95% CI=0.20-0.56, p=0.001). None of haplotype combinations of exon 3 (*Tyr113His*) and exon 4 (*His139Arg*) polymorphisms showed modulation of risk for EC. In association of clinical phenotype with genotypes, sub-grouping of patients based on anatomical location of tumor predicted that patients with exon 3 *His113His* and *Tyr113His* genotype were at high risk for developing upper and middle third tumor in EC (OR 4.4, 95%CI=1.0-18.5, p=0.04; OR 2.5, 95%CI=1.3-5.0, p=0.005). The frequency of exon 4 *His139Arg* genotype was significantly lower in EC patients with lower third location as compared to controls (14.8% vs. 36.3%, p=0.02). Gene-environment interaction of *EPHX1* genotypes with tobacco, alcohol and occupational exposures did not appear to modulate the cancer susceptibility.

Conclusion: Exon 3 *Tyr113His* genotype was associated with higher risk of EC, its clinical phenotypes and anatomical location. However, exon 4 *His139Arg* genotype exhibited low risk for EC as well as its clinical characteristics.

BP4

CYP3A5 GENOTYPE INFLUENCES THE BLOOD CONCENTRATION OF TACROLIMUS IN RESPONSE TO INHIBITION BY KETOCONAZOLE

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There is marked heterogeneity in blood concentrations of tacrolimus following standard body-weight-based dosing. Differences in hepatic and intestinal cytochrome P4503A activity have been postulated as contributing to this problem, probably through linkage with an SNP in the CYP3A5 gene. It has been shown that patients with at least one CYP3A5*1 allele achieved twofold lower dose-normalized tacrolimus blood concentrations compared with CYP3A5*3/*3 homozygote. We investigated the role of this polymorphism on response to inhibition of the enzyme activity by ketoconazole (used to bring down the cost of therapy). The dose-normalized blood concentrations of tacrolimus 2 weeks after starting 100 mg/day of ketoconazole in 136 previously stable renal transplant recipients were related to CYP3A5 genotypes determined by polymerase chain reaction followed by restriction fragment length polymorphism analysis. We found that presence of even one copy of the *1 allele was associated with significantly higher tacrolimus trough concentration compared to those who carried two *3 alleles. Those carrying one *1 allele required significantly lower tacrolimus dose to achieve similar therapeutic levels. We conclude that CYP35 genotype is a major factor in determining the dose requirement for tacrolimus in response to metabolic inhibitors, and determination of the CYP3A5*1/*3 genotype could be used to predict the tacrolimus dose requirement. This finding has major implications in optimizing immunosuppressive therapy in cost-constrained settings.

BP5

ENDOTHELIN-1 (ET-1) LYS198ASN VARIANT AND ALTERED CIRCULATING ET-1 LEVELS PLAY A ROLE IN DEVELOPMENT OF PREECLAMPSIA

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Preeclampsia, characterized by development of new-onset hypertension, proteinuria and edema during late pregnancy, is associated with systemic endothelial dysfunction. The maternal syndrome may be caused by an imbalance of endothelium derived vasodilators and vasoconstrictors. Variation in the sequence of gene encoding the vasoconstrictor peptide endothelin-1 (ET-1) that leads to substitution of lysine at 198 position with asparagine (Lys198Asn) is associated with increased ET-1 systemic levels. We investigated the relationship between ET-1 Lys198Asn variant and expression of ET-1 in placentae at mRNA and protein levels by RT-PCR and immunohistochemistry respectively and the circulating ET-1 levels using ELISA in preeclamptic (PE), gestational hypertensive (GH) and normotensive pregnant (N) women. The Asn variant was encountered more frequently at genotypic [OR (PE v N): 2.68 (95%CI 1.21 – 5.91); OR (GH v N): 2.85 (95%CI 1.29 – 6.29)] and allelic [OR (PE v N): 1.4 (95%CI 0.02 – 84.9); OR (GH v N): 1.43 (95%CI 0.02 – 85.2)] levels in PE and GH compared to N group. Placental ET-1 mRNA was significantly reduced in PE and GH compared to N group (PE v N: $p < 0.000$; GH v N: $p < 0.000$). A significant decrease was observed in ET-1 expression in placental endothelium (PE v N: $p < 0.01$) but not in villous trophoblasts and extravillous trophoblasts. Circulating ET-1 levels were also significantly elevated in PE compared to GH and N group (PE v GH & N: $p < 0.0001$). Overall, the circulating ET1 levels were higher in those bearing even one copy of the Asn variant at this locus. We conclude that women carrying ET1 198Asn variant are predisposed to endothelial activation that leads to release of ET-1 in circulation and contributes to the development of hypertension during pregnancy.

BP6

INHERITED ALOPECIA AND ECTODERMAL DYSPLASIA IN FAMILIES FROM SOUTHERN PUNJAB AND NORTHERN PAKISTAN

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Abstract:

Alopecia (AP) is a rare autosomal recessive disorder characterized clinically by total or partial hair loss soon after their birth and the development of papular lesions of keratin-filled cysts over extensive area of body. The hypohidrotic and anhydrotic ectodermal dysplasia (ED) is a genetic disorder characterized by the absence or hypoplasia of hair, teeth and eccrine sweat gland. More than 200 distinct clinical forms have been reported so far. The X-linked hypohidrotic ectodermal dysplasia (XLED) is the most common form of ED. In this study, twelve consanguineous families of alopecia and seven with ectodermal dysplasia were ascertained from Southern Punjab and Northern area of Pakistan having multiple affected members. The mode of inheritance was determined from the pedigree analysis which was autosomal recessive in the four families of AP. These pedigrees were excluded for linkage to the ten most common loci reported using the microsatellite markers. One AP family was linked to the type 1 keratin genes (17q21.2locus) while remaining are in the process of exclusion analysis. Four pedigrees with ED were excluded to all known loci whereas one was linked to the type II keratin (KRT) genes (12q13.13 locus). Two ED pedigrees are in the process for exclusion analysis. The excluded families will be subjected to genome wide scan through SNP (Single Nucleotide Polymorphism) using Affymetrix 250K array system to find the homozygous regions for identification of the novel loci. The validity of the data will be tested through statistical analysis such as LOD (log of odds).

BP7

STUDY OF DEMOGRAPHIC PATTERN AND CLINICO- INVESTIGATIONAL PROFILE OF CHILDREN WITH β -THALASSEMIA MAJOR IN NORTH INDIA

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Introduction : The demographical profile of thalassemia is invaluable for knowing the true burden of disease and to be able to plan screening programmes, necessary for the control of the disease.

Aims & Objectives : To evaluate the socio-demographic pattern and the clinico-investigational profile of patients with β -thalassemia major.

Materials and Methods : Case files of 964 patients diagnosed as Thalassemia major between 1982 & 2007 were retrieved from the medical record department. Data pertaining to demographic profile, symptomatology and investigations was recorded on a pre-designed proforma. This data was subsequently pooled for statistical analysis.

Results : The mean age of presentation was 17.2 months (SD \pm 19.9 months). 503 (52%) patients presented before 12 months of age. There were 688 (71.4%) boys and 276 (28.6%) girls. 47.4% patients belonged to the Khatri and the Arora communities which constitute about 15% of the population in this part of the country. A large percent of patients [457 (47.4%)] had ancestors who were first/second generation immigrants from Pakistan. A family history of thalassemia was elicited in 176 (20%) patients. Parental education up to high school and college was seen in about 35% and 25% cases respectively.

Summary & Conclusions: Thalassemia is a public health problem which needs to be addressed aggressively in our country. This disease is more prevalent amongst the Khatri / Arora communities of Punjab.

Families show a significant level of literacy of up to high school and graduate level.

This information can help in formulating public awareness education programmes at school / college levels for the control of this dreaded disease.

BP8

TOLL LIKE RECEPTOR 4 AND PROINFLAMMATORY MEDIATORS IN PROSTATE CANCER

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Introduction: Prostate cancer (CaP) is the most common cancer in males and accounts for 4% of all cancers in developing countries. Inflammatory response has been associated with CaP. Toll like receptor 4 (TLR-4) is a key trigger of the innate immune response in the development of various inflammatory diseases. Hence in this study role of TLR-4 in CaP was unraveled.

Objective: To study polymorphism and expression of TLR-4 in prostate cancer.

Methods: CaP tissues were confirmed histopathologically and blood samples were collected from CaP, BPH (benign prostate hyperplasia) patients. TLR-4 gene was amplified and PCR-RFLP analysis was performed using restriction enzymes Nco1 and Hinf1. Expression of TLR-4, COX-2, IL-1 β , IL-6 was checked from tissue of prostate cancer and BPH.

Result: Histopathological analysis of the samples shows the presence of lymphocytes, neutrophils, eosinophils, mononuclear cell which are the markers of inflammation. Allelic variation in TLR-4 gene was observed in CaP (25.25%), BPH (17%), and control (14.29%) samples at Nco1 restriction site. Presence of variant allele was significantly higher in prostate cancer as compared to control ($X^2=6.31$, $p=0.011997$). However variant allele with respect to Hinf1 was not found to be significant. Expression of TLR-4, COX-2 and IL-1 β was higher in patients with CaP in contrast to IL-6 expression which was more in BPH.

Conclusion: Histopathological analysis and presence of elevated level of proinflammatory TLR-4, COX-2 and IL-1 β , suggests a role of inflammation in CaP. Individuals with TLR-4 variant allele with respect to Nco1 appear to be at high risk for CaP. We are further investigating inflammatory response in cell line.

BP9

GENETIC LINEAGE AND SUSCEPTIBILITY TO TUBERCULOSIS OF SAHARIYA TRIBE OF CENTRAL INDIA

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Introduction: The Sahariya tribe is a primitive tribal group, mainly populated in the North-West region of Madhya Pradesh, Chattisgarh and Rajasthan, (India). The tribal group is distributed in many districts of the M.P. Despite being a significant part of our genetic lineage, no genetic study has yet been carried out, either on the origin of this tribe or the diseases, infectious or otherwise, with which they are inflicted upon. The past and our recent surveys on the health conditions of this tribe revealed significantly high incidence of pulmonary tuberculosis in adults.

Objectives: In this study we have made a preliminary analysis on the maternal and paternal lineages of this tribe with an objective to identify possible genetic factor, which might be predisposing them to increased mycobacterial infections, if any.

Methods: A demographic survey was made from six *Sahariya* villages (N= 355). Peripheral blood samples were collected after well informed consent of donors. DNA markers including 55 Y-SNPs in 69 Y-chromosomes and 4Y-STRs in 80 Y-chromosomes were analysed by PCR-

based genotyping method. In addition, mitochondrial haplo-groups were assigned to 200 samples after HVRI sequencing using ABI prism sequencer. TLR1 (rs4833095) and TLR 2 (arg 753gln) polymorphism were studied as a potential candidate gene for TB susceptibility.

Results: The Y-SNP and STR analyses showed that the tribe has a high frequency of R1a1* haplo-group, that is, 0.18, than R2, O and L haplogroups, which were found to be least in the tribe. The mitochondrial haplogroup M (G10398A) was also found to be at a very high frequency. The TLR1 SNP (rs 4833095) screening revealed that 20.6% of cases (sputum positive TB patients) have mutant allele C (homozygous) instead of T (wild type allele), while 58.8% controls were heterozygous for this mutation.

Conclusion: R1a1* Y-haplogroup may have evolved independently in India. High frequency of M-haplogroup in tribe suggests tribe's primitive maternal lineage. TLR1 and TLR2 polymorphism also revealed interesting results that will be discussed during presentation.

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BP10

PREVALENCE OF CFTR GENE MUTATIONS IN THE POPULATION OF THE STATE OF UTTAR PRADESH: A PRILIMINARY REPORT

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Introduction: Mutation in CFTR gene results in Cystic Fibrosis an autosomal recessive single gene disorder most common among Caucasians (1:2500) but is found in all racial and ethnic groups, characterized by defective Chloride ion transport which leads to mucosal obstruction in various organs having Secretory epithelial cells notably in lungs and pancreas resulting in chronic lung infection and chronic pancreatitis.

Objective: To find out the gene frequency of CFTR gene mutations (Delta F508, G542X and G551D) in the population of the state of Uttar Pradesh.

Methods: A total 300 subjects were screened for Delta F508, G542X and G551D mutations by using Amplification refractory mutation system (ARMS) PCR method.

Results: After screening of 300 individuals we found three heterozygous for Delta F508, four heterozygous for G542X mutations. Two subjects with mild bronchial disorders were found

positive for G551D mutation. In two families prenatal diagnosis was performed and the one fetus was found homozygous for G542X mutation.

Conclusion: However it is difficult to interpret the gene frequency in the state of Uttar Pradesh due to small sample size. The preliminary data indicates the presence of CFTR gene mutation in the population of Uttar Pradesh.

BP11

ANALYSIS OF AMNIOTIC FLUID SPECIMENS FOR COMMON CHROMOSOME DISORDERS USING INTERPHASE FLUORESCENT IN SITU HYBRIDIZATION

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Introduction: Aneuploidies involving chromosomes 13, 18, 21, X and Y constitutes more than 80% of all live born chromosomal abnormalities associated with birth defects. In high risk pregnancies Fluorescent in situ Hybridization (FISH) on uncultured amniocytes with chromosome specific probes was described for rapid prenatal diagnosis of aneuploidies.

Material and Methods: 132 amniotic fluid samples were collected for interphase FISH analysis. All the probes were directly labeled with fluorescent molecules. Fluorescent signals were observed under a microscope. A minimum of 100 nuclei with defined hybridization signals were counted for each probe.

Results: 132 amniotic fluid samples were received for FISH analysis. The average age of mothers and their gestational ages were 31 years and 17.5 weeks respectively. Triple test screening was positive in 40% of the women followed by advanced maternal age and ultrasonographic abnormalities. Interphase FISH was performed on 128 specimens whereas 4 samples were rejected because of blood contamination. Aneuploidy was identified in 10 out of 132 specimens. Five cases of trisomy 21, 3 cases of trisomy 18 and 1 case of monosomy X were detected. In addition, one case showed 10% mosaicism for trisomy 21. Initially 4 (5.3%) samples were uninformative due to technical reasons but gave acceptable scoring signals when reanalyzed.

Conclusions: This study demonstrated that Interphase FISH is a rapid and reliable technique for the enumeration of chromosome number in uncultured amniocytes. Clinicians can use it for making early decisions necessary for the management of high risk pregnancies ultimately saving patients from anxiety and psychological stress.

BP12

MECP2 GENE MUTATIONS IN INDIAN PATIENTS WITH RETT SYNDROME

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Introduction: Rett syndrome (RS) is an X-linked progressive neurodevelopmental disorder that almost exclusively affects girls, and is one of the most common causes of mental retardation in females. Mutations in X-linked methyl-CpG-binding protein 2 (*MECP2*) gene, located on chromosome Xq28, have been found to be a cause of RS. Mutations are found in 70% - 80% of patients with classical RS and in less than 50% of patients with atypical RS.

OBJECTIVE The present study aimed to investigate frequency and type of mutations of *MECP2* gene in Indian patients with Rett syndrome.

METHODS: A systematic analysis of exon 2-4 of *MECP2* in 25 sporadic patients with RS was performed by polymerase chain reaction (PCR), single strand conformation polymorphism (SSCP), Restriction fragment length polymorphism (RFLP), followed by sequencing.

RESULTS: Five mutations were identified in 12 (48%) of 25 patients. Most of the mutations were nonsense mutations; p.R168X was found in 5 (20%) of 25 patients; p.R270X was found in 3 (12%) of 25 patients, p.R255X was found in 2 (8%) of 25 patients, p.T158M and p.D156E were found in 1 (4%) of 25 patients, respectively.

CONCLUSION: The results of this study indicated that mutational spectrum of RS in Indian population is similar to studies from around the world. The p.R168X is the most common mutation in Indian patients followed by p.R270X, p.R255X, p.T158M, p.D156E and these were hot spot mutations in *MECP2* gene of Indian patients with RS.

BP13

STATUS OF HFE GENE MUTATIONS (C282Y & H63D) IN CHRONIC LIVER DISEASES PATIENTS: AN INDIAN SCENARIO

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Abstract

Introduction: Hereditary hemochromatosis (HH) is an autosomal recessive single gene disorder characterized by defective iron metabolism which leads to iron deposition in various parenchymal organs notably the liver resulting in liver cirrhosis.

Objective: To find out the presence of HFE gene mutations (C282Y & H63D) in chronic liver disease (CLD) patients and healthy controls in Indian population

Methods : A total 496 CLD patients with different subgroups (HBV cirrhosis = 74, HCV cirrhosis = 50, Alcoholic cirrhosis with Hepatitis = 38, Alcoholic cirrhosis w/o hepatitis = 92 & Cryptogenic cirrhosis = 242) and 502 healthy controls was screened for C282Y and H63D mutation by using PCR-RFLP method.

Results : Except a single compound heterozygote (C282Y/H63D), none of the subject was found positive for C282Y mutation. No significant difference was found between the overall H63D allele frequency of patients and controls (5.95 & 4.58 respectively). However a highly significant H63D allele frequency was found among two patients subgroups i.e. HBV cirrhosis (10.82) [p = 0.002] and Alcoholic hepatitis with cirrhosis patients (11.84) [p = 0.006] . The other subgroups of CLD patients have comparable frequency with respect to controls. No association was found between primary iron overload and HFE mutations.

Conclusion: This is the first study showing that H63D heterozygosity may be an increased risk factor for HBV cirrhosis and alcoholic hepatitis patients in Indian population.

BP14

ASSOCIATION OF NEURONAL AND ENDOTHELIAL NITRIC OXIDE SYNTHASE GENE POLYMORPHISM WITH ASTHMA AND AUTONOMIC FUNCTIONS

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Introduction: Nitric oxide (NO) is physiologically linked to asthma because of its being a neurotransmitter for bronchodilator inhibitory nonadrenergic and noncholinergic nerves, and also its role in modulating autonomic balance. NO is thought to generate and perpetuate airway inflammation thereby causing airways hyper-reactivity. Animal gene knock out models have clearly indicated the role of nitric oxide synthase (NOS) genes in hyper-reactivity and inflammation asthma. Recently, a polymorphism of the NOS1 gene C5266T and NOS3 gene G894T have been found to be associated with asthma in several populations. We investigated

the association between asthma trait, autonomic balance and genetic polymorphisms in NOS1 and NOS3 in a North Indian population.

Methods: 137 asthmatic patients were tested for the presence of NOS1 C5266T and NOS3 gene G894T polymorphisms. Spectral analysis of heart rate variability was performed to quantify sympathetic and parasympathetic autonomic activity. Autonomic parameters were compared between asthmatics and controls. Dependence of autonomic balance on genotype was analyzed using general linear model with asthma as a covariate.

Results: Subjects with variant alleles of NOS1 and NOS3 had a greater but insignificantly higher risk for asthma. Asthmatics exhibited higher parasympathetic tone. Subjects with NOS1 polymorphism had a higher parasympathetic tone as reflected by higher high- frequency heart rate variability independent of asthma.

Conclusions: This study reveals association of C5266T polymorphism in NOS1 gene with asthma phenotype and higher parasympathetic tone. This polymorphism might be increasing the risk of asthma by inciting inflammation in concert with higher background parasympathetic tone.

BP15

MUTATION ANALYSIS AND PRENATAL DIAGNOSIS OF MEGALENCEPHALIC LEUKODYSTROPHY

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Introduction: Megalencephalic leukodystrophy with subcortical cysts (MLC) is an autosomal recessive disorder, characterized by megalencephaly, seizures, ataxia and mental deterioration. It is caused by mutation in the *MLC1* gene. It is found commonly in Agarwals in India and the common mutation is a homozygous change (c.135_136insC) in exon 2 of *MLC1* gene.

Objectives: Our aim is to do the mutation analysis of MLC patients and to develop strategy for prenatal diagnosis.

Methods: Blood samples from 24 MLC patients were collected and genomic DNA was extracted. Exon 2 of *MLC1* gene was amplified and the PCR product (171bp) was restricted by enzyme *Mwo*1. In the affected patient, the 171 bp band will be restricted into two DNA bands (144bp and 27bp) in polyacrylamide gel electrophoresis, while the wild one will show only one unrestricted band of 171bp. In two families prenatal diagnosis (PND) using CV was also offered.

Sequencing was done to confirm results of PND. In patients negative for this common mutation, CSGE was done. Sequencing was done to identify the mutation.

Results: Among 24 patients, 20 patients were homozygous and one was heterozygous for c.135_136insC mutation. Three patients showed homozygous mutation C959A in exon 11 of *MLC1* gene. Out of two CV DNA sample, one was homozygous while other was heterozygous for the common mutation.

Conclusion: The results of c.135_136insC common mutation in MLC patients were consistent with previous reports. C959A mutation found in exon 11 of *MLC1* gene seems to be other novel and causative mutation of MLC in Indian patients.

BP16

RISK OF E-SELECTIN S128R POLYMORPHISM TOWARDS GENETIC PREDISPOSITION OF CORONARY ARTERY DISEASE IN NORTH INDIAN POPULATION

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Introduction: E-Selectin (CD62E) is surface glycoprotein molecule involve in adhesion of circulating leukocyte to activated endothelium which play an important role in inflammation process and inflammation is one of the earliest event in pathogenesis of atherosclerosis. The E-selectin belongs to a family of structurally related selectin molecule; selectin E, selectin P & selectin L participate in endothelial leukocyte adhesion.

Objective: Aim of the present study to determine the influence of S128R polymorphism of E-selectin gene in north Indian CAD patients.

Method: We have genotyped the S128R polymorphism by PCR-RFLP in n=226 Angiographically documented CAD patients and n=215 healthy individuals as control.

Result: The study reviled that R allele is significantly associated with CAD patients (R allele frequency 9.9% in CAD patients vs 5.8% in control P value = 0.034, OR = 1.79, (95% CI = 1.05 – 3.07).

Conclusion: SR genotype of E-selectin S128R polymorphism is an independent risk factor for CAD. In addition to classical risk factors, genetic predisposition may thus play an important role in pathogenesis of CAD.

BP17

PREVENTION OF HOMOZYGOUS BETA THALASSEMIA BY CARRIER SCREENING AND PRENATAL DIAGNOSIS IN NORTHERN PART OF THE INDIA

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Introduction: Beta thalassemia is a heterogeneous group of autosomal recessive disorder that results from reduced or absent production of β globin chain. The disorder has markedly high frequency in Mediterranean area, Middle East, Indian subcontinent and Southeast Asia. We report results of 3 years pilot screening programme coupled with prenatal diagnosis directed to the prospective prevention of homozygous β thalassemia in India

Objective: The screening programme took two approaches: firstly testing of the extended family members of diagnosed thalassemia major cases and offered them genetic counseling & prenatal diagnosis. Secondly screening of all referred cases of anemia to rule out thalassemia carrier status and offered them premarital genetic counseling & prenatal diagnosis as per need.

Methods: Prenatal detection is achieved by DNA analysis on chorionic villus samples obtained by ultrasound guided trans abdominal or trans vaginal procedures. The ARMS-PCR technique and sequencing were used for identification of mutations in DNA samples carriers and high –risk couples

Results: We screened 548 family members among them 200 couples were found where both the partners were carriers, out of them 112 were opted for antenatal diagnosis as women were pregnant at the time of screening. The DNA diagnosis revealed 24% affected fetus, 54.4% carriers & 21.4% fetuses as normal.

However, on screening of 2287 cases to rule out the cause of anemia, 243 cases as thalassemia trait, 48 cases as thalassemia major, 57 as β thalassemia with structural variants [E β , S β & D β] & 71 with structural variants were observed. During this period 20 couples were eligible for prenatal diagnosis after counseling. DNA diagnosis on 20 CVS samples revealed; 5 affected fetus, 9 carriers & 6 normal.

Conclusion: Hence, during this period screening of 2835 individuals could result in prevention of 32 thalassemia major babies. Thus the carrier screening and PND program in the country has prevented the birth of thalassemia homozygous by 1.13 %.

BP18

A RAPID MOLECULAR SCREENING METHOD FOR FRAGILE-X SYNDROME AT REFERRAL GENETIC CENTRE - CREMERE EXPERIENCE.

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Introduction: Fragile X syndrome (FXS) characterized by a fragile site on chromosome Xq27.3 region is the 2nd most common genetic cause of mental retardation. Hence early diagnosis is important for prevention, carrier detection & effective genetic counseling. It results from expansion of CGG repeats at 5' UTR of FMR1 gene causing methylation of promoter gene, thus silencing it.

Objectives: To screen intellectually disabled (ID) children with unknown etiology for FXS using cytogenetic and molecular techniques and to compare these methods for FXS screening.

Methods: The referred children suggestive of FXS features were selected. A high-resolution G-banding karyotype using folate deficient media was carried out to study chromosomal aberration by light microscopy. The genomic DNA was amplified by PCR and gel electrophoresis was performed to detect FRAX-A mutation.

Results: After clinical genetic examination of 394 cases with IQ <70, 135 (34.2%), were selected for cytogenetic & molecular screening. Of 135, 18(13.33%) revealed fragile site on X chromosome. However, 18.75% were positive for FXS by PCR method (9/48). The comparison of two methods was available in 9; one of these was found negative on karyotyping but was positive on molecular screening. The PCR technique had an advantage over cytogenetic will be illustrated through family studies.

Conclusion: The high-resolution banding in clinically diagnosed FXS cases has a chance of missing about 11% patients. Thus, PCR method was found more accurate and rapid test for screening ID children at referral genetic centers, thereby reducing the national burden of genetic disorders in India.

BP19

IS ADVANCED MATERNAL AGE A CRITERIA FOR SCREENING INDIAN PREGNANT WOMEN?- CHROMOSOMAL PROFILE IN 280 DOWN SYNDROMES

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Introduction- Down Syndrome is the most common genetic cause of intellectual disability (ID) caused by an extra chromosome 21, and distinguishing physical features. Non-disjunction of chromosome 21 (more common in mothers above 35 years age) during meiosis in the maternal or paternal gametes leads to this chromosomal constitution in the embryo. Hence the aneuploidy screening became a practice.

Aim-To determine the maternal age as a high risk factor in Indian women with Down syndrome.

Materials and methods- The clinically diagnosed Down syndrome cases were studied recording maternal age. Karyotyping was conducted using peripheral lymphocytes culture for G-Banding and High-Resolution banding of chromosomes.

Result- Chromosomal analysis was conducted in 280 Down Syndrome patients. Free trisomy 21 was prominent (91%), followed by translocations (3.6%) and mosaicism (5 %). A detailed case history available in 154 mothers revealed 23.0% advanced (>35 years) maternal age, while the majority (70%) were between 21-35 years age. The rest were 6%.

Conclusion- The common health policy in developed countries of considering the advanced maternal age as a high risk factor does not hold true in our country because most pregnant women are in younger age group. They also carry the risk of non –disjunction (70%) which is evident by their Down syndrome children. Therefore effective antenatal screening strategy for prevention of Down syndrome/ aneuploidies is recommended in all pregnant women. Rapid prenatal DNA diagnostic techniques and pregnancy monitoring is the need of today as a preventive approach for reducing the genetic burden in India. –

BP20

SCREENING AND DIAGNOSIS OF PROPIONIC ACIDEMIA BY GAS CHROMATOGRAPHY / MASS SPECTROMETRY IN CRITICALLY ILL BABIES

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Introduction: Propionic acidemia (PA) is an autosomal recessive organic acidopathy due to deficiency of enzyme Propionyl-CoA Carboxylase (PCC) complex. Mutations in the PCCA and

PCCB genes coding for two subunits of PCC disrupt its function causing accumulation of propionyl-CoA and other compounds that cause brain damage.

Objective: To reliably diagnose PA in high-risk cases for early intervention, & to detect its incidence using advanced method of Gas chromatography/Mass spectrometry (GC/MS).

Method: The urine samples of 2699 cases were treated with urease, denatured, dried and then treated with N, O-bis-trimethylsilyltrifluoroacetamide and trimethylchlorosilane, injected into GC & detected by MS using MILS method for the specific markers of PA.

Results: Screening of high-risk patients detected 844 (31%) cases of Inborn Errors of Metabolism (IEM). Total 33% (278/844), were of amino and organic acidopathies; and PA was observed in 16 (6.23%) cases with male female ratio 1.28:1, and mean age 7.4 months. Clinical history in 7/16 PA patients revealed birth asphyxia, coma, low birth weight, meconium aspiration, hyperbilirubinemia, developmental delay, and history of neonatal death in family (14.3%). Neonatal convulsions, prematurity, and infection were in 28.6%, dysmorphic features 43%, & respiratory distress in 57 % patients. Urine sample of one patient collected after death by puncturing urinary bladder could also detect PA.

Conclusion: GC/MS using non-invasive MILS method was found specific, sensitive, and reliable for diagnosis of PA with incidence of 1 in 160. Screening for organic disorders in NICU babies with advanced techniques can prevent early death/ morbidity with effective genetic counseling.

BP21

GALACTOSEMIA- POTENT CANDIDATE FOR NEW BORN SCREENING IN INDIA!

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Introduction: Galactosemia is an inherited autosomal recessive disorder of galactose metabolism that causes failure to thrive, vomiting, hepatic dysfunction, cataracts, speech abnormality and mental deficiency. It is due to enzyme deficiency of galactokinase, galactose-1-phosphate uridylyltransferase or uridine diphosphate galactose-4-epimerase in the affected

patients. Frequently associated septicemia often misleads the clinician & delays the diagnosis, resulting into mortality / morbidity.

Objective: To reliably diagnose Galactosemia in critically ill neonates & infants using GC/MS method for effective genetic counseling & prevention of the disability, & also to know its incidence in India.

Methods: The urine samples of high-risk cases were screened by GC/MS using MILS method for specific biochemical markers of Galactosemia - galactose, galactitol and galactonate.

Results: Out of 2699 cases, 844 (31.00 %) were diagnosed with metabolic abnormality; of these 55 (6.51%) showed sugar metabolism disorders. Galactosemia was found in 24/55 (43.64 %) patients with age range 1 day to 2 ½ years and mean age 6 months. The male to female ratio was 3:1. A significant proportion of cases were associated with hyperbilirubinemia (16.67 %) and hepatomegaly (16.67 %).

Conclusion: Using advanced technology, the higher incidence (1:113) of Galactosemia was noticed indicating it as the candidate for New Born Screening in India. Though enzyme assay is necessary for differential diagnosis, accurate detection of Galactosemia helped in starting early intervention & therapy, thereby preventing death or developmental delay. A simultaneous screening of multiple categories of disorders used in this study offered better option to the clinician in treating the patients appropriately.

BP22

DATA ON SERVICES AVAILABLE TO HIV/AIDS PATIENTS IN TWO TERTIARY CARE HOSPITALS OF PESHAWAR.

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Objectives: To appreciate services available to HIV/AIDS patients in two tertiary care hospitals of Peshawar.

Settings: Khyber teaching hospital (KHT) and postgraduate medical institute, hayatabad medical complex Peshawar (PGMI, HMC).

Duration: From September 2005 to February 2006.

Material and Methods: A questionnaire was designed in accordance with the objectives of the study. Questionnaire contained information's about, the medical services available to HIV/AIDS patients, clinico-pathological aspects of the disease, risk behaviors, preventive and precautionary measures, diagnosis and treatment of the HIV/AIDS.

Results: A total of 100 medical faculty members were selected, 64% from KHT and 36% from HMC. Voluntary counseling and testing (VCT) is available in these centers (76%). Preliminary tests for HIV testing done on site (70%). Pre and post-test counseling (68%) and space to ensure privacy (60%) were available. No confidentiality testing, trained counselors and quality control for HIV testing recorded. Services for special and vulnerable groups were only for pregnant ladies (90%). Distribution of laboratory tests or medical procedures that are part of the routine HIV-treatment/follow-up was: complete blood count (100%), liver function tests, Hbs Ag/HCV Antibodies (100%), syphilis serology (68%), toxoplasmosis serology (38%) and MBT culture (65%) etc. No facilities for CD4cell count, viral loading testing, cryptococcal Ag, anergy testing and PAP smear recorded. Drugs used for treatment for opportunistic infections associated with HIV/AIDS were available (74%). Drugs used for treatment of malignancies associated with HIV/AIDS were available on site/city (56%). Antiretroviral availability (available on order only) recorded was: nucleoside reverse transcriptase inhibitors (NRTI)(n: 16%) and protease inhibitors (n: 4%).

Conclusion: Medical services available in two tertiary care centers of Peshawar are not satisfactory. There is need for further actions, more collaboration, leadership, community involvement, UN assistance and government support to improve the quality of life of people living with HIV/AIDS.

BP23

ASSOCIATION OF FACTOR V LEIDEN MUTATION, ANGIOTENSINOGEN M235T, ANGIOTENSIN-CONVERTING ENZYME I/D, METHYLENE TETRAHYDROFOLATE REDUCTASE C677T AND APOLIPOPROTEIN B C2388T POLYMORPHISM WITH PRE-ECLAMPSIA IN NORTH INDIAN WOMEN

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INTRODUCTION: Preeclampsia is considered to be a multifactorial and multisystemic disorder with a genetic predisposition. Alterations in renin-angiotensin system are considered to play a significant role in the pathogenesis of the disease.

OBJECTIVE: The contribution of genetic factors to preeclampsia has been well documented. As till date no genetic analysis has been done on Indian population, thus we performed an hospital based case-control study on North Indian women to investigate the association of preeclampsia with the F5 Leiden mutation, AGT M235T, ACE I/D, MTHFR C677T, and APOB C2388T polymorphisms.

METHODS: DNA was extracted from whole blood of 100 preeclamptic pregnant women and 200 normotensive healthy pregnant women. Samples were analysed for the polymorphisms using PCR amplification of known allelic variants.

RESULTS: our results showed that T235 allele of AGT, I allele of ACE and A1691 allele of F5 has odd's ratio of 1.13, 1.33, and 2.09 in preeclamptic women respectively.

CONCLUSION: The results indicate that AGT T235, ACE I and F5 A1691 alleles act as the genetic risk factors for preeclampsia in our selected population.

BP24

GSTM1 AND GSTT1 GENE POLYMORPHISM IN BONE MARROW FAILURE SYNDROMES.

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INTRODUCTION: Bone marrow failure syndromes (BMFS) comprise of aplastic anemia and myelodysplastic syndromes (MDS). BMFS are classified as constitutional, acquired secondary and acquired primary subtype, the latter being diagnosed in the absence of former two types. The definition of exact etiology is important for the obvious reasons of proper management, family screening and genetic counseling etc. Constitutional factors contribute to BMFS in nearly 1/3 of young patients, according to western literature. The acquired factors include drugs, chemicals, toxins, infections (mainly viral) etc. Such data on etiological factors is largely not available from Indian subcontinent. The exact frequency of etiological factors, especially constitutional, is not reported from any part of globe. Most of the genetic predisposition syndromes (chromosomal instability syndromes, constitutional chromosomal disorders, neurofibromatosis 1 and toxin modifying enzymes variants etc.) require specific diagnostic tests

and many (like Fanconi anemia) require modifications in the treatment strategy. BMFS are a leading cause of mortality and morbidity in haematological practice.

MATERIALS AND METHODS: The PCR genotyping for GSTM1 and GSTT1 was undertaken in 92 aplastic anemia patients, 40 MDS patients and 96 normal healthy controls.

RESULTS: Normal GST genotype was detected in 47 aplastic anemia patients, 13 MDS patients and 82 control individuals. GSTT1 null genotype was found in 9 aplastic anemia patients, 10 MDS patients and 8 control individuals ($p > 0.05$). Prevalence of GSTM1 null genotype was found to be significantly more in 22 aplastic anemia patients and 17 MDS patients as compared to 6 control individuals ($p < 0.01$). GSTM1 and GSTT1 null genotype was found in 4 aplastic anemia patients.

CONCLUSIONS: GSTM1 null genotype most likely contributes to the causation of BMFS in our patient population.

BP25

CLINICAL PROFILE OF PATIENTS WITH DOWN SYNDROME: PILOT STUDY AT A SINGLE TERTIARY CARE CENTER.

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INTRODUCTION: Down syndrome is the most common trisomy with incidence of 1 in 600 to 800 live births. Modern approach to management of DS involves a team oriented approach addressing a multiplicity of issues including medico-surgical, psychosocial and reproductive issues.

AIMS AND OBJECTIVES: To study the clinical profile of DS presenting at our Institute.

MATERIAL AND METHODS: The clinical profile and the karyotype were recorded in a predesigned performa and the data was subsequently pooled and analyzed.

RESULTS: The mean age at presentation was 17 ± 13.8 months and the male: female ratio was 1:1. Karyotypic anomaly in all the patients was trisomy 21. The mean birth weight and age at conception was 2400 ± 122 grams and 28.8 ± 2.6 years respectively. History of prior abortion was present in 37.5% mothers. One or more phenotypic features of DS were present in 93.7% patients. Digital anomalies were seen 31.2% patients. Cardiac anomalies and hypothyroidism was present in 2 and 1 patients respectively. MTHFR polymorphism was present in 44.4% mothers. The numbers were small hence further analysis was not performed.

CONCLUSIONS: Majority of the patients (56.2%) presented in infancy. History of miscarriages and MTHFR polymorphism were present in 37.5% and 44.4% mothers respectively. Compared to studies in control subjects the incidence of MTHFR polymorphism was higher in our study. There was no increased incidence of perinatal events in DS. No other CMF was specifically associated with DS. The long term outcome remains modest in the developing countries due to lack of supportive care.

BP26

METHYLENETETRAHYDROFOLATE REDUCTASE MTHFR C677T AND A1298C POLYMORPHISMS: ASSOCIATION WITH RISK FOR CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

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Introduction: Methylenetetrahydrofolate reductase (MTHFR) is an essential enzyme in the metabolism of folate; it plays a central role in converting folate to methyl donor for DNA methylation. The presence of polymorphisms that reduce the activity of MTHFR has been linked to the process of development of acute leukemia. Recently, Methylenetetrahydrofolate reductase (MTHFR C677T and A1298C) mutations were discovered to be associated with childhood acute lymphoblastic leukemia (ALL), as well as colon cancer, lymphoma, esophageal and stomach cancer. Therefore, it was hypothesized that the MTHFR polymorphisms are associated with the risk of childhood ALL in the Indian population.

Objective: To determine the association between MTHFR gene polymorphisms (C677T and A1298C) and childhood Acute Lymphoblastic Leukemia.

Patients and Methods: A case-control study was conducted in 96 pediatric patients diagnosed with ALL and 251 healthy controls for MTHFR C677T, and 96 pediatric ALL patients and 80 healthy controls for MTHFR A1298C polymorphisms. DNA was extracted from peripheral blood lymphocytes using phenol-chloroform method. Polymerase chain reaction (PCR) amplification followed by Hinf1 and MbolI restriction digestion was used to determine the MTHFR C677T and A1298C genotypes respectively.

Result: The genotype 677TT was found to be associated with increased risk (OR = 7.89 with a 95% CI of 0.02-3408.44) whereas the genotype 1298CC was linked with a decreased risk (OR = 0.89 with a 95% CI of 0.46-1.74) of developing childhood ALL in Indian population.

Conclusion: Significant association between MTHFR variants and risk of ALL were observed. Our findings suggest that the MTHFR C677T and A1298C gene variants may have an influence on the susceptibility to pediatric ALL in our sample population.

BP27

PEDIATRIC DISORDERS OF SEX DEVELOPMENT: A STUDY AT A SINGLE TERTIARY CARE CENTRE.

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INTRODUCTION: Disorders of sex development (DSD) are congenital conditions in which development of chromosomal, gonadal or anatomical sex is atypical. Modern treatment of children with DSDs involves a team-oriented approach but many patients avoid interactions with healthcare providers and special effort is necessary to optimize long-term surgical, medical, and psychological management.

AIMS AND OBJECTIVES:

- To study the clinicophenotypic correlation of pediatric DSD.
- To assess the correlation of karyotype with the presenting phenotype.

METHODOLOGY: The data regarding clinical profile, investigations and diagnosis of the pertinent patients was entered in a predesigned performa. Subsequently the data was pooled and statistical analysis was performed.

RESULTS: The mean age at presentation was 31.3±9 months (range: 1 day to 144 months). Majority of patients (87.9%) presented before 5 years of age and 36.2% presented in infancy. The Karyotype was 46XY and 46XX in 74.1% and 20.7% patients respectively and 74.1% patients were reared as males. Karyotype was significantly associated with gonadal palpability ($p=0.001$) and evidence of Mullerian structures ($p=0.0001$). Penoscrotal hypospadias, small phallus and clitoral hypertrophy were present in 29.3%, 12.1% and 5.2% patients respectively. Palpable gonads correlated well with evidence of testicular tissue but only 20% of these had

gonadal dysgenesis. Phenotypic class I and III had 34 (58.6%) and 20 (34.5%) patients respectively with only 2 patients in class II and one each in class IV and VI. Congenital adrenal hyperplasia (CAH) was the commonest (36.2%) cause of DSD in our study.

CONCLUSIONS: DSD are associated with social stigma and neglect leading to delayed presentation and diagnosis as well as treatment. The commonest cause of DSDs is CAH, a potentially treatable condition. Phenotypic classification along with karyotype provides a useful and practical guide to investigations and management.

BP28

MUTATIONAL ANALYSIS OF THE HFE, TFR2 AND HEPICIDIN GENES ASSOCIATED WITH PRIMARY IRON OVERLOAD IN NORTHERN INDIANS

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INTRODUCTION: Hereditary Haemochromatosis is a genetic disorder that results from the disruption of the mechanism that regulates iron absorption. It is commonly encountered in northern Europeans and is due to the C282Y mutation of the HFE gene in 85-90% cases. This disorder is uncommon in India.

AIMS: To determine the prevalence of primary iron overload and HFE genotypes in normal controls as well as chronic liver disease (CLD) patients.

MATERIALS AND METHODS: Hundred controls and 238 patients of CLD (23: Alcoholic liver disease, 18: viral cirrhosis, 138: cryptogenic cirrhosis and 59: non alcoholic steatohepatitis) were analyzed for iron parameters and DNA analysis for three HFE mutations (C282Y, H63D, and S65C) by PCR-RFLP. HFE, TFR2 and Hfeclidin genes were fully scanned by DNA sequencing in all patients who were consistent with the diagnosis of primary iron overload.

RESULTS: Seventeen cases of primary iron overload were identified from 238 patients with CLD. No patient or individual showed C282Y /S65C mutations. The prevalence of H63D was 12% in normal individuals, 14.2% in 238 patients and three out of seventeen cases of primary iron overload showed heterozygosity for H63D (which was not statistically significant). One patient and one normal individual were homozygous for 63D but they did not show iron overload. DNA sequencing of the HFE gene in all the cases of primary iron overload revealed one patient

with compound heterozygosity for H63D and T218I. Other patients showed normal HFE gene sequence. Seven patients of primary iron overload showed HFE splice site mutation (IVS2+4T/C; 2 homozygous, 5 heterozygous); Haplotyping of H63D showed it to be present on haplotype 6 (CTA) which confirms the single origin in our population. This pattern is similar to the European and dissimilar to Sri Lankan population. The TFR2 gene was fully scanned by DNA sequencing and showed no mutation in the coding region but only one polymorphism in intron 17 [IVS17 (-418)] was found. The Hfeclin gene was sequenced and no mutation or polymorphism was found.

CONCLUSION: This study confirms that the primary iron overload in Indian patients is uncommon and is of the non-HFE type, which is different from the Europeans. It also appears that the HFE, TFR2 and Hfeclin genes are not involved with primary iron overload in Indians.

BP29

EXPRESSION OF c-kit IN HUMAN RETINOBLASTOMA: A POTENTIAL MOLECULAR TARGET FOR THERAPY

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Introduction: Retinoblastoma is the commonest eye tumor in childhood. Treatment modalities for retinoblastoma include enucleation, chemotherapy, cryotherapy, laser photocoagulation and radiotherapy. However, the presence of multiple side effects along with multidrug resistance has fostered the development of a new class of cytotoxic agents. The target of these agents is individual gene that has altered expression in tumor cells. c-kit (CD117) is a tyrosine kinase receptor, which plays an important role in cellular mechanisms, such as differentiation, proliferation, regulatory processes and signal transduction. The overexpression of c-kit has been reported in many different types of human malignancies. c-kit has emerged as a promising therapeutic target after the recent development of specific tyrosine kinase inhibitors.

Objectives: To assess the expression of c-kit in human retinoblastoma and correlate its expression with histopathological features.

Methods: c-kit reactivity was evaluated by immunohistochemistry in twenty eyes enucleated from retinoblastoma patients. Degree of tumor differentiation, choroid invasion and optic nerve

infiltration were determined. c-kit expression was further correlated with these tumor characteristics.

Results: c-kit immunopositivity was observed in eleven tumors. The expression of c-kit significantly correlated with histopathological features of a worse prognosis including optic nerve and choroid invasion.

Conclusion: Our results show that expression of c-kit in human retinoblastoma is associated with tumor characteristics. A deeper understanding of c-kit molecular pathway in human retinoblastoma may help to design better and more specific therapeutic modalities including specific tyrosine kinase inhibitors.

BP30

THE ASSOCIATION OF FACTOR V LEIDEN AND ANTIPHOSPHOLIPID ANTIBODY SYNDROME WITH RECURRENT PREGNANCY LOSS

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Introduction: Unexplained recurrent pregnancy loss (RPL) as defined by occurrence of three or more consecutive spontaneous abortions is a significant distressing public health problem affecting 1-5% of women of reproductive age group. Fewer than 50% of cases have definitive cause. A large body of evidence obtained during past several years suggests a potential association of inherited and acquired thrombophilias in causation of poor gestational outcome by causation of placental vascular abnormalities.

Factor V Leiden (FVL) is the most common form of inherited thrombophilia. Antiphospholipid antibodies (APA) are recognized as one of the most important causes of acquired thrombophilia.

Objectives: The purpose of this study was to evaluate the association of FVL and antiphospholipid antibody syndrome (APLA) with recurrent first trimester abortions and unexplained late trimester (second/third trimester) loss.

Methods: We enrolled 90 women with ≥ 2 unexplained early trimester abortion and 90 females with ≥ 2 unexplained late trimester loss (second/ third trimester) loss. Thirty age matched women with at least one prior normal delivery and no prior history of fetal loss or thrombosis were considered for the control group. These women were tested for FVL mutation by DNA analysis and for presence of APA- Lupus Anticoagulant (LA) and Anticardiolipin

antibodies (ACL) by clotting screen, kaolin clotting time, dilute Russel viper venom time and ELISA respectively.

Result: Four out of 90 (4.44%) patients with history of recurrent first trimester loss tested positive for presence of ACL antibodies while 9/90 (10%) patients with history of recurrent second trimester loss tested positive for presence of ACL antibodies. 6/90(6.66%) patients with history of recurrent second trimester loss tested positive for presence of LA antibodies. Amongst a total of 180 patients studied only one patient with history of late fetal loss carried the FVL mutation in heterozygous form i.e. 0.55%. This is in contrast to overall prevalence of 3.03% of FVL mutation in same population which was obtained after screening 330 normal individuals.

Conclusions: Our data shows that APLA is a significant contributing factor towards recurrent pregnancy losses but our data falls short of showing significant association between FVL mutation and recurrent pregnancy loss.

BP31

CORRELATION OF RED CELL INDICES AND HbA₂ LEVELS WITH DIFFERENT MUTATIONS IN BETA THALASSEMIA TRAITS

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Introduction: Individuals with beta thalassemia trait (β TT) carry the genetic trait for beta thalassemia and are phenotypically asymptomatic. Screening for β TT can be performed using red cell indices and raised HbA₂ (>4%) levels. In this study, we have tried to correlate the red cell indices and HbA₂ levels with the five mutations prevalent in India, together with -88 (C \rightarrow T) and Cap+1 (A \rightarrow C).

Objectives: To analyze the red cell indices in cases of β TT and correlate them with the common Indian mutations as well as -88 (C \rightarrow T) and Cap+1 (A \rightarrow C).

Methods: Laboratory data of all cases with a diagnosis of β TT were retrieved from the database of the Department of Haematology. The haematological parameters (hemoglobin levels, RBC count, MCV, MCH, MCHC and RDW) were obtained from automated blood cell counters along with HbA₂ levels using HPLC. Patients with haemoglobin levels below 10 gm% were excluded from the study to minimize patients with concomitant iron deficiency anemia. The β -thalassemia mutations were analyzed using PCR-based Amplification Refractory Mutation System.

Results: A total of 433 cases were included in the study [116 with IVS I-5 (G → C), 83 with 619 base pair deletion, 58 with Fr41 / 42 (-TTCT), 66 with Fr8 / 9 (+G), 43 with IVS I-1 (G → T), 42 with -88 (C → T) and 33 with Cap+1 (A → C)]. The hemoglobin levels ranged from 10 g% to highest of 16.3g%. The differences between the mean hemoglobin values were significant when Cap+1 (A → C) was compared to IVS I-5 (G → C), 619 bp deletion and Fr41 / 42 ($p = 0.021$, 0.04 , 0.035 , respectively). Similarly, mean MCV and MCH values were affected minimally (c.f. normal values) in cases with -88 and Cap +1 mutation in contrast to the other categories ($p=0.000$). The mean HbA₂ level in the category of Cap+1 was 3.56 ± 0.43 as compared to all other groups ($p = <0.05$). The variations amongst RBC counts, RDW and MCHC were however not found to be significant. We have documented earlier that the -88 (C→T) is seen almost exclusively in *Jat Sikhs* of Punjab amongst Asian Indians

Conclusion: Patients of β thalassemia trait with Cap+1 (A → C) and – 88 (C → T) mutations have better hematological profile as compared to other mutations prevalent in India. Thus a basic set of investigations and information of the caste might serve as a useful guide to predict the expected mutation and thus help in further DNA analysis.

BP32

RATE OF HETEROZYGOSITY DETERMINATION BY STUDYING POLYMORPHISMS IN FACTOR VIII GENE TO DETECT THE INFORMATIVENESS OF VARIOUS POLYMORPHIC MARKERS IN NORTH INDIANS.

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Introduction: Haemophilia A is an X linked, recessively inherited bleeding disorder which results from deficiency of procoagulant factor VIII, affecting males invariably. This disease affects approximately 1 in 5000 males worldwide. Mutations in factor VIII gene are known to be associated with Haemophilia A. The factor VIII gene is a very large gene of 180 Kb having 26 exons and 25 introns. It is located at the long arm of X chromosome (Xq28). Several intragenic and extragenic markers have been identified to detect the polymorphisms in the factor VIII gene and hence determine the heterozygosity associated with it. The frequency of heterozygosity will help us to detect the carriers of the disease and subsequently for prenatal diagnosis of haemophilia A, thereby preventing the birth of an affected individual with haemophilia A.

Aim: To determine the rate of heterozygosity by studying polymorphisms in factor VIII gene in healthy females from north India to plan which RFLP/VNTR is more informative.

Materials and methods: This study was conducted on healthy females. Genomic DNA was isolated from peripheral blood leucocytes by phenol-chloroform extraction method. The samples were screened for intron 19 (Hind III), intron 18 (Bcl 1) and intron 22 (Xba1) polymorphisms by conventional PCR followed by restriction digestion with relevant enzymes (PCR-RFLP) method.

Results: Out of 94 healthy volunteers, 40 showed heterozygosity for Hind III, thereby showing 43% heterozygosity. Thirty seven were heterozygous out of 74 volunteers screened for Bcl 1 showing 50% heterozygosity and 24 were heterozygous out of 58 volunteers for Xba 1, thereby showing 41% heterozygosity. The combined cumulative heterozygosity of all the three markers in the common volunteers was 65%.

Conclusion: The compound approach for determining the heterozygosity in factor VIII gene polymorphism proved to be a very efficient, cost effective and informative strategy. It can also be further used for carriership determination for factor VIII mutation in north Indian population. In this study, we found that Bcl 1(50%) was the most informative marker, followed by Hind III (43%) and finally Xba 1 (41%) in north Indian population. Moreover this method is very fast compared to the traditional Southern blot method for determining the heterozygosity as the results are expected in 24 hours and cheap in comparison to sequencing. Using this strategy and combining amelogenin primers (for sex determination) we have performed antenatal testing in 3 families.

BP 33

STUDY OF GENETIC POLYMORPHISMS IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION

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Introduction: Individual thrombotic tendency increases the risk of Acute Myocardial Infarction (AMI), especially in young adults. Several pro-thrombotic factors that may influence the individual thrombotic risk have been identified.

Objective: To determine the association between polymorphisms of genes encoding hemostatic factors and acute myocardial infarction.

Methods: This comparative study involved 108 patients who had survived a first AMI at an age ≤ 40 years, 155 patients who had survived a first AMI at an age ≥ 60 years and 50 healthy subjects matched for age and geographic origin. EDTA–anticoagulated whole blood was collected, DNA was extracted and polymorphisms were detected by PCR amplification of nine genes {Factor V Leiden (1691G/A), Gp1 β HPA-2 (1018C/T) and VNTR, GpIIIa 1565T/C, GpIa 807C/T, FVII R353Q (10976G/A) and HVR4, ACE I/D and AT1R 1166A/C} with subsequent restriction enzyme digestion & gel electrophoresis.

Results: FV Leiden (1691G/A), Gp1 β HPA-2 (1018C/T) and FVII R353Q (10976G/A) were significantly associated with increased risk of AMI in both patients ≤ 40 years and ≥ 60 years. Rest of the 6 gene polymorphisms: GpIIIa 1565T/C, Gp1 β VNTR, GpIa 807C/T, FVII HVR4, ACE I/D and AT1R 1166A/C were not associated with increased or decreased risk of AMI in patients ≤ 40 years and ≥ 60 years.

Genotypes	OR (Controls Vs Pts ≤ 40 yrs) (95% CI)	OR (Controls Vs Pts ≥ 60 yrs) (95% CI)
FV Leiden (1691 G/A)	3.4 (0.406 - 28.376)	1.2 (0.138 - 11.582)
Gp1 β HPA-2 (1018 C/T)	2.8 (0.338 - 24.603)	3.0 (0.373 - 24.450)
FVII R353Q (10976 G/A)	28.8 (3.831 - 216.853)	38.3 (5.156 - 284.448)
GpIIIa 1565 T/C	1.0 (0.434 - 2.470)	0.9 (0.418 - 2.209)
Gp1 β VNTR	0.2 (0.132 - 0.545)	0.2 (0.103 - 0.404)
GpIa 807 C/T	1.2 (0.349 - 3.940)	0.9 (0.267 to 2.892)
FVII HVR4	0.9 (0.377 - 2.199)	0.7 (0.303 - 1.680)
ACE I/D	1.2 (0.467 - 3.483)	0.9 (0.335 - 2.433)
AT1R 1166 A/C	1.2 (CI 0.524 - 2.901)	1.2 (0.544 - 2.791)

Conclusion: FV Leiden, Gp1 β HPA-2 and FVII R353Q polymorphisms have an important association in patients with AMI in our population.

THALASSEMIA INTERMEDIA IN VARANASI REGION (EASTERN UP AND ADJOINING AREAS OF NEIGHBORING STATES).

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The present study is based on 697 cases of thalassemia and hemoglobinopathies detected between 2000 and 2007. 105 cases presenting as thalassemia intermedia (TI) comprised 45% of the symptomatic cases of thalassemia. Of these 29 cases were of homozygous beta thalassemia, 27 of heterozygous beta thalassemia, 24 of E-beta thalassemia, 16 of S-beta thalassemia and 9 had HbH disease. The high percentage of TI cases can be attributed to the significant presence of abnormal Hemoglobins E and S, as evident by the above data. 11 cases of alpha thalassemia point to significant presence of alpha thalassemia in the region, further modifying the thalassemia phenotype. Initial genotyping of 74 subjects indicates wide heterogeneity in beta globin gene mutations. IVS1-5 remains the commonest with 33% of cases while 25% constitute the other 4 common mutations (IVS1-1, CD8/9, CD41/42 and 619bp deletion). The remaining 33% show a wide spectrum of mutations which could be contributing to the wide variation in TI phenotype.

This presentation highlights the importance of TI in the region with its varied clinical manifestations. A comprehensive study of various primary, secondary and tertiary genetic and environmental modifiers is indicated for further classification of TI cases.

P1

A PRELIMINARY STUDY OF THE SPECTRUM OF β -GLOBIN GENE MUTATIONS AMONG THALASSAEMIA PATIENTS IN IRAQ

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Introduction: Although β -thalassaemia is one of the most common genetic disorders in Iraq with an estimated carrier prevalence of 4.6%, to date molecular data on the patterns of causative disease mutations have been very limited.

Objectives: To determine the spectrum of mutations present in patients clinically diagnosed with β -thalassaemia attending the Ibnalbaldy Thalassaemia Centre in Baghdad.

Methods: Blood samples were obtained from a group of 100 unrelated male and female patients aged 7-12 years. DNA was obtained by phenol/chloroform extraction and the β -globin gene was amplified in three overlapping fragments using the polymerase chain reaction, followed by direct DNA sequencing in an ABI 3730xl 96-capillary automated DNA sequencers with Big Dye terminator chemistry.

Results: Four mutations accounted for more than 80% of the thalassaemic chromosomes analysed: β^0 IVSII 1 G>A (HBB:c.315+1G>A), IVS-I-110 (G->A) β^+ (HBB:c.93-21G>A), β^+ IVSI 6 T>C (HBB:c.92+6T>C), and the frameshift mutation at Cd36/37 (-T) β^0 (HBB:c.112delT). Each of these mutations is common in the Mediterranean region and among Arab populations.

Conclusion: These initial results indicate that analysis of DNA samples for the four common β -globin gene mutations would provide good coverage for β -thalassaemia carrier screening in the study population. However, given the ethnic sub-divisions in the Iraqi population, the strong tradition of marriage within clan and tribal boundaries, and an overall consanguineous marriage rate of 40-50%, it seems probable that more detailed community-specific studies will be required in the future.

P2

OCULAR INVOLVEMENT IN SICKLE CELL HEMOGLOBINOPATHIES

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Introduction: Sickle Hemoglobinopathies, being a systemic disorder, have pronounced involvement in eyes.

Material & Method: 74 number of sickle positive cases who attended the Eye Department of V.S.S.Medical College, Burla during the year from June 2002 to May 2005 have been included in this study. Detail clinical, pathological & ophthalmologic examinations were done in all the cases.

Results: The hemoglobin patters were 45(AS),21(SS) & 8(ASF).The ocular changes were bilateral ptosis with external ophthalmoplegia in 1 case ,conjunctival vascular changes in 27 cases & retinal involvement in 22 cases.

Discussions: The ocular involvement in Sickle Hemoglobinopathies in India is minimal in comparison to sickle cell HbC disease found elsewhere.

P3

PRENATAL DIAGNOSIS FOR β -THALASSEMIA BASED ON ETHNIC DIFFERENCES: A STEP TOWARDS ITS CONTROL IN A DEVELOPING COUNTRY.

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Introduction: Thalassemia major (TM) is an autosomal recessive disorder where the parents are asymptomatic beta thalassemia carriers (β TT). On screening 1500 voluntary blood donors in our region we found a β TT prevalence of 3.5%, majority occurring in the khatri-arora caste. Though >250 mutations are described world wide, few mutations occur in an ethnic background which results in antenatal programmes to be effective. Objectives: To analyze the spectrum of beta thalassemia mutations in north Indians and apply this information for prenatal diagnosis.

Methods: Detailed proforma was filled for each patient and relevant investigations including automated blood counts, HPLC, hemoglobin electrophoresis were done. Specific PCR-ARMS

for beta thalassemia mutations on DNA extracted from peripheral blood genomic DNA by chloroform phenol method (or Qiagen columns for amniotic fluid or CVS) was carried out.

Results: Analyzed of 1087 beta thalassemia alleles [835 TM & 252 Thalassemia intermedia (TI)] showed the common 5 Indian mutations was found in 81.4% alleles; commonest being IVS 1,5 (G-C) in 24.7%, followed by 619 bp deletion in 15.3%, Fr 8/9 (+G) in 15.8%, IVS 1,1 (G-T) in 14.3% and Fr 41/42 (-TTCT) in 11.3%. The uncommon β^{++} mutations -88 (C-T) and Cap+1 (A-C) were found in 10.9% alleles. Rare mutations constituted 6.4% alleles & 1.3% alleles remained uncharacterized. This data helped 282 pregnancies in 245 women who underwent amniotic fluid/CVS based prenatal diagnoses. In 24.5% cases the fetuses were normal, 47% cases were \square TT, 23.7% were TM and in 4.6% we were unable to distinguish between normal or \square TT or maternal contamination.

Conclusions: The common five Indian mutations occurs in 81.4% alleles in north Indians. Prenatal diagnosis is a feasible option in India to prevent the birth of infants with thalassemia major.

P 4

UTILITY OF FAMILY STUDIES IN DIAGNOSING ABNORMAL HAEMOGLOBINS/THALASSEMIC STATES INDETERMINATE ON SCREENING WITH HIGH PERFORMANCE LIQUID CHROMATOGRAPHY.

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Introduction: Currently HPLC is the most popular modality used for screening abnormal haemoglobins /thalassemic states. However, some of the compound heterozygous states cannot be clearly differentiated from homozygous forms. For eg. Homozygous E Vs E- β thalassemia, Homozygous S Vs S- β thalassemia & thalassemia major cases especially in first 6 months of life. HPLC alone may not be useful in these cases and further investigation with family studies or genetic analysis can help us determine the exact genotype.

Objectives: To resolve all indeterminate case on HPLC screening with the help of family studies and confirming the results by genetic analysis.

Methods: In our 10 years experience with HPLC at Sir Ganga Ram Hospital, 50 such cases were solved by us with the help of family studies and the diagnosis was confirmed further by genetic analysis.

Results: In 100% of cases, we noted that the diagnosis obtained by family studies was commensurate with that obtained by DNA analysis.

Conclusion: In centers, which do not have the facility for genetic analysis, family studies by the HPLC can be equally useful.

P 5

STUDY OF BETA GLOBIN GENE CLUSTER HAPLOTYPE IN SICKLE CELL DISEASE PATIENTS OF WESTERN ORISSA AND CORRELATION WITH VARIOUS HEMATOLOGICAL PARAMETERS

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Introduction: Sickle cell disease(SCD) is a common genetic disorder in Western Orissa (India), with a gene frequency of 15% in certain caste Hindus.

Objective: To study of beta globin gene cluster haplotype of SCD patients of Western Orissa and correlate with various hematological parameters and fetal hemoglobin concentration.

Method: Beta globin haplotypes of 124 SCD was done by RFLP with restriction enzymes HincII ϵ , XmnI 5' γ G, HindIII γ G, HindIII γ A, HincII $\psi\beta$, HincII 3' $\psi\beta$, RsaI 5' β , Avall β , HinfI 3' β . HbF concentration estimated by HPLC (Biorad). CBC done by automated cell counter (Sysmex).

Results: One hundred five (84.7%) patients were found to be homozygous for typical Asian Indian haplotype [+ + + - + + + -] / [+ + + - + + + -] ([Asian]/ [Asian]), 3 (2.4%) were [Asian]/ [Senegal] ([+ + + - + + + -] / [- + + - + + + -]) and rest 16 were atypical haplotypes. In the 105 (84.7%) cases of typical Asian Indian haplotype the hematological parameters(units) (Mean \pm SD) (range) was found to be HbF(%) (22.6 \pm 7) (5.5 - 40.9), Hb(g%) (8.6 \pm 2.2) (3.6 - 13), MCV (fl) (89.76 \pm 11.4), MCH (pg) (25.1 \pm 3.89) (17-40.7). In the Asian / Atypical haplotype group (13%) HbF (22.9 \pm 10.8) (8.2 - 40.2), Hb(g%) (10.1 \pm 2.49) (5.7 - 14.4), MCV (fl) (72.8 \pm 10.2) (70.3 - 96.2), MCH (pg) (21.4), (18.5 - 30.7).

Conclusion: Of the 124 cases of Sickle Cell Disease 105 (84.7%) were typical Asian haplotype, 3 (2.4%) were Asian/ Senegal haplotype and 16 (13%) were Asian / Atypical haplotype. The mean HbF concentration was 22% and was comparable in all the groups.

P 6

COMBINING BIOINFORMATICS AND POPULATION GENETICS IN THE STUDY OF THALASSAEMIA

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Introduction: The public database HbVar (<http://globin.cse.psu.edu/globin/hbvar/>), contains information on 1,324 molecular variants, including 979 haemoglobin variants and 395 thalassaemia entries. Although much research has been conducted on molecular and clinical aspects of thalassaemia and other haemoglobin variants, to date the underlying contribution of population genetics has received little attention.

Objectives: To investigate examples of geographical and regional variation in β -thalassaemia and other haemoglobinopathies in order to identify the strengths and weaknesses of the available data in HbVar, and their applicability to community- and population-based studies.

Methods: Information on haemoglobin variants listed in HbVar was initially examined and data on Indian, Pakistani and Italian individuals and communities were selected and abstracted. These data were then subjected to Venn analysis and GIS mapping.

Results: Few data were available for the Indian sub-continent. The initial analysis also revealed a lack of clear delineation of regional and community identification in the information listed for Indian and Pakistani individuals, thus limiting its application to population genetics analysis. To overcome these limitations, a method of analysis was developed for mapping Italian data on haemoglobin variants using Google Map technology.

Conclusions: In countries such as India and Pakistan, with multiple geographical, ethnic, language and social subdivisions, it is probable that many haemoglobin variants and thalassaemia mutations are community-specific. The ability to pinpoint regional and community variants would greatly assist genomic screening and prenatal diagnosis programmes. To fulfil

the aims of the Asian Thalassaemia Network, the collection of appropriately detailed population data is urgently needed.

P 7

COMMUNITY GENETIC APPROACH IN THE PREVENTION OF BETA THALASSAEMIA IN EASTERN INDIA

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Introduction: Eastern India harbors high beta thalassaemia and HbE trait frequencies with a significant number of beta thalassaemia syndrome births requiring regular blood transfusion and medical management. Considering the clinical severity and consequent mortality associated with the affected phenotypes, it becomes very much essential to initiate a prevention program directed towards awareness generation, mass screening and premarital counseling with respect to the haemoglobinopathies. Earlier studies have reported a strong ethnic exclusiveness for beta-globin mutations, which reflects the geographical localization and pattern of population movements at a macro level. However, there is still a need to understand the complex pattern of distribution of the mutations among different ethnic groups delineated by a proper designed population study.

Objectives: The Survey has initiated a massive population-screening program for haemoglobinopathies in the eastern part of West Bengal, India, with the following objectives: (1) To initiate a mass awareness program for beta thalassaemia (2) To initiate mass screening and (3) To ascertain the beta thalassaemia mutation frequencies in the studied area.

Methods: Altogether 4020 unrelated individuals aged 13 to 30 years from the coastal south 24 Parganas district of West Bengal have participated in the mass screening drive for beta thalassaemia under the Community Genetics Extension Program undertaken by the Anthropological Survey of India during 2006 – 2007. The methods used for the present study were osmotic fragility, CBC, HPLC , ARMS-PCR and sequencing of beta-globin gene.

Results and Conclusion: The level of awareness in the studied area was very poor. About 85% of the people in the studied area do not have a brief idea about the difference between thalassaemia trait and disease. Altogether 13.7% of the subjects were identified to carry

abnormalities of hemoglobin in one or other forms. The IVSI-nt5-G>C was found to account for about 75% of all the mutations, followed by cd41/42 (-TCTT), cd15 (G>A) and others. The approach undertaken by the Survey would be a benchmark for future reference and intervention programme in controlling haemoglobinopathies in India.

P 8

GENOTYPIC AND PHENOTYPIC CORRELATION OF THALASSEMIA MAJOR & THALASSEMIA INTERMEDIA WITH BETA THALASSEMIA MUTATIONS AND *XMN-1*^G γ POLYMORPHISM

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Introduction: β thalassemia patients are phenotypically categorized as thalassemia major (TM), thalassemia intermedia (TI) & β thalassemia trait (β TT). *Xmn-1*^G γ polymorphism is a known factor which increases HbF production.

Aims & Objectives: To detect the frequency of *Xmn-1*^G γ polymorphism in patients with TM & TI.

Materials & Methods: A total of 90 cases were selected, 45 each of TM & TI. Complete blood counts, *Xmn-1*^G γ polymorphism & β thalassemia mutation studies were performed. A detailed clinical history was obtained regarding age of 1st presentation, timing of 1st blood transfusion & hemoglobin at presentation. Genomic DNA was extracted from peripheral blood leucocytes by chloroform –phenol method. ARMS –PCR was performed to detect the β thalassemia mutation. *Xmn-1*^G γ polymorphism was done using PCR based RFLP. Specific primers were used as per the reference.

Results: The 45 TM patients had presented before 3 yrs of age. TI patients had the age of presentation ranging from 3.5 -27 yrs. The 45 patients of TM were categorized on the basis of *Xmn-1*^G γ polymorphism as +/+ (4/45; 9%), +/- (5/45; 11%) & -/- (36/45; 80%). For TI, the categorization was 29/45 (+/+; 44%), 9/45 (+/-; 20%) & 16/45(-/-; 36%). The cases which had received blood transfusion were excluded from analysis for complete blood cell counts. It was

observed that the patients with +/- had a higher average Hb (9.2 gm%) as compared to -/- (5.27gm %). The commonest mutation noted for TM was IVS1,5 (G → C) homozygous (17/45) & IVS 1,1 (G → T) homozygous (23/45) for TI patients.

P 9

COMMON HEREDOFAMILIAL OCULAR DISORDERS IN WESTERN ORISSA

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Introduction: Many congenital and developmental ocular disorders are due to heredofamilial transmission.

Material & Methods: A detail family history, obstetric history & clinicopathological examinations were done in these cases observed by the author from 2002 to till date in Eye Department of V.S.S.Medical College, Burla.

Result: A total 189 number of heredofamilial and congenital ocular disorders were observed by the author during the study period. The cases were corneal dystrophy-2, AniridiaPeter's anomaly-1, Glaucoma-30, cataract-20, Retinoblastoma-15, Colour-blindness-9, Retinitis Pigmentosa-25, Ocular albinism-4, Age related Muscular degeneration-10, Pathological Myopia-35, Coloboma-10, Anophthalmos-2, Microphthalmos-20 & others-6.

Discussions: The cases of Retinitis pigmentosa, pathological myopia had definite history of heredofamilial transmission where as others appeared to be stray cases.

P 10

INVESTIGATION OF THROMBOPHILIA DURING PREGNANCY AND PUERPERIUM: A STUDY FROM NORTH INDIA

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Pregnancy, from implantation to parturition, results in a hypercoagulable state due to physiological changes in hemostasis. We studied some of the genetic risk factors for thromboembolism to identify the etiology of thrombosis in pregnant and postpartum women. Over a 10 year period, we encountered 102 women (out of 1800 patients; 5.6%) who were investigated for thrombosis associated with pregnancy. Eighty one were post partum, 16 were intrapartum and five presented with recurrent pregnancy loss. The commonest presentation was deep vein thrombosis in 54, followed by cerebral venous thrombosis in 34, Budd-Chiari syndrome in 7 and seven with arterial thrombosis. PCR-RFLP for Factor V Leiden (FVL G1691A), Prothrombin G20210A (PT G20210A), Methylene tetrahydrofolate reductase C677T (MTHFR C677T) and A4070G polymorphism in exon 13 (B domain) of factor V was carried out on genomic DNA. Comparison with indigenous normal controls and Odd's ratio with 95% confidence interval was calculated. The prevalence of heterozygosity for FVL was 6.9% (7/102) versus 3% (10/330) in normals and the OR was 2.35 (95% CI 0.87-6.36). All the 450 individuals (cases and controls) screened for PT G20210A showed the wild allele. The prevalence of MTHFR C677 was 31.3% versus 24.7% in 251 controls (OR of 1.57 with 95% CI 0.94-2.63). The OR of A4070G polymorphism was 2.03 (95% CI 1.04-3.95) on screening 86 cases and 250 controls.

Conclusion: We found that FVL, MTHFR C677 and A4070G polymorphism are encountered in our population with an approximately two fold increase in risk for thrombosis in pregnant woman and PT G20210A is uncommon in our population.

P 11

CYTOGENETIC ABERRATIONS IN BAD OBSTETRIC HISTORY AND INFERTILITY CASES

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Bad Obstetric History (BOH) as indicated by recurrent spontaneous abortions and infertility cases have varied etiology including infections with Rubella, Toxoplasma, hormonal imbalance or genetic factors. The genetic basis of infertility and bad obstetric history is poorly understood. Chromosomal rearrangements, Reciprocal or Robertsonian translocations, are observed in 2-

4% infertility or recurrent pregnancy loss cases, in either partner. With a view to understand chromosomal aberrations in BOH and infertility cases, we analyzed 1260 cases constituting 630 referred couples, (January 2006 to December 2007). Conventional karyotyping – GTG banding, on 72 hour PHA stimulated peripheral blood cell cultures, using Cytovision 3.1 software, was the method of choice. We observed chromosomal translocations in 4% (25 of 630 couples), with 18 reciprocal translocations and 7 Robertsonian translocations. The aberrations were randomly distributed in all the chromosomes in 1 – 2 individual cases, with the exception of chromosome 13 involved in 5 cases, chromosome 6 in three cases, and no associated aberrations in chromosomes 17 and 19 in any of the BOH cases. Further, two females and one male showed mosaicism of sex chromosome, and a single female with inversion of the long arm of chromosome 10. Identification of the varied genes in these cases using CGH microarrays, may help in understanding the genotype-phenotype correlations. The identification of chromosomal abnormality in the BOH cases, was essential in genetic counseling to the couples, and risk estimation in subsequent pregnancies

P 12

CHROMOSOMAL ABNORMALITIES IN INTELLECTUAL DISABILITY WITH EPILEPSY

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Introduction: Intellectual disability (IQ<70) is a heterogeneous group of disorders, and 27-30% of patients manifest epilepsy. Chromosomal abnormalities have been increasingly recognized as significant clinical problem in children with intellectual disability & epilepsy. However, systematic studies are scanty in Indian population. Hence, a chromosomal study was undertaken in intellectually disabled children with epilepsy.

Objective: To detect structural and numerical chromosomal abnormalities in children with intellectual disability & epilepsy.

Methods: Total 111 cases (Male- 85; Female–26) were screened by high-resolution G-banding karyotype using peripheral blood lymphocytes & studied by light microscopy.

Results: Male preponderance was evident (M-30; F-2). The common type of seizure was GTC 82% (90/111), followed by myoclonic 5.4% (6/111), complex partial 3% (3/111) & other types of seizures. Genetic Syndromes had high association with chromosomal imbalances & observed

in 5 cases viz Fragile X (1), Angelman (1), Cornelia de Lange (2) & Dandy walker syndrome (1). The autosomal abnormality 54% (17/32) consisted of 9qh+ (n=3), 21p+ (n=2), 22ps+ (n=3), 15p- (n=1), inv.9 & 17 (n=1), & two cases with translocations. The sex chromosomal abnormality was 46%(15/32) in terms of Meta Y (n=2), Yqh+ (n=6), Yqh- (n=6) & fra. Xq27 .3 (n=1).

Conclusion: The association between specific chromosomal abnormality & epilepsy is important for molecular analysis to identify the genes. A high resolution banding helped to detect even smaller chromosomal imbalances in the present study. Hence, it is recommended to undertake high-resolution karyotype in epilepsy with intellectual disability for effective genetic counseling and estimating recurrence risk for prevention.

P 13

NEUROIMAGING FINDINGS IN CHILDREN WITH INTELLECTUAL DISABILITY & EPILEPSY

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Introduction- Epilepsy with intellectual disability (IQ<70) includes a specific etiology, associated neurological abnormalities, genetic background, idiopathic and/ environment factors. Many epileptic conditions with characteristic clinical and brain abnormalities are categorized as syndromes. The accurate & early diagnosis is important as it helps in prognosis, management, & genetic counseling.

Objectives- A prospective study was undertaken to correlate neuroimaging findings of children with intellectual disability and epilepsy for characterizing structural anomalies of the brain and to evaluate the diagnostic yield of neuroimaging.

Methods- A detailed neurological examination, with family history was conducted in total 98 patients with intellectual disability and epilepsy (M-70; F-28) referred to Clinical Genetic OPD, followed by brain CT/ MRI.

Results- Male preponderance was noticed. EEG was available in 61% (60/98) patients with 87% showing abnormality; of these 81% showed bilateral abnormality. Brain CT (59%) was more frequently conducted for financial reasons, than MRI (46%). The definite genetic syndromes with epilepsy were identified in 17 children. The brain structural abnormalities revealed were developmental migration defects (38 %), global atrophy (27 %), white matter degeneration/metabolic (15%), PVL with birth asphyxia (14%), heteropia (5%), agenesis of corpus callosum (4%), and hippocampus mesial temporal sclerosis (3%).

Conclusion- Severe brain abnormalities were correlated with severe (42.86%) and moderate retardation (30%) when compared with the children with mild retardation (14%). Congenital malformations were predominantly indicating the underlying genetic factor, and therefore all cases with epilepsy and intellectual disability need brain imaging for further molecular analysis to identify new epilepsy genes.

P 14

PRECONCEPTIONAL GENETIC COUNSELLING IN CONGENITAL METABOLIC DISORDERS- PSYCHOSOCIAL AND EMOTIONAL ISSUES IN INDIAN SCENARIO

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Introduction: Majority of congenital metabolic disorders (CMD) are inherited, autosomal recessive with asymptomatic carrier parents. Diagnosis of a CMD causes enormous emotional stress, and parents need personal support to explain the nature, recurrence risk and prenatal options. Knowledge about therapy and prognosis of a particular metabolic condition during a preconceptional period allows the couple to plan the next pregnancy,

Objective: To assess psychosocial, emotional issues of parents & other family members affected with CMD, against the background of religious, ethnic and socio-cultural diversity with customs like consanguinity and endogamous marriages prevalent in Indian population.

Method: After the first child with a particular metabolic disorder accurately diagnosed by Gas Chromatography/ Mass Spectrometry, 23 couples approached for preconceptional counseling. Inheritance nature, 25 % recurrence risk and both parents carrying defective genes was explained. A questionnaire of 10 points was given to couples covering the psychosocial, emotional and financial issues who underwent prenatal diagnosis. Newborn Screening after 1 week & follow-up confirmed the normal baby.

Results: The survey revealed cost factor (60%), consent & knowledge of prenatal tests (90%), expecting reliability (92%), Govt. help in care (81%), inheritance & consanguinity awareness (50%), & social stigma (15%).

Conclusion: Preconceptional genetic counseling plays a crucial role in families having CMD, and genetic support groups should be formed for such parents for exchange of knowledge and

awareness about the specific inherited disorders. Though cost factor was a major constraint, lack of knowledge about the inheritance of disease was news to parents.

P 15

MARKER AND RING CHROMOSOMES IN TURNER SYNDROME VARIANTS

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Globally, Turner Syndrome has been reported in 1 of 2500 live female births. Fifty percent of all patients with Turner syndrome have 45,X karyotype, while the remaining 50% exhibit mosaicism and other sex chromosomal abnormalities. In case of mosaic karyotype, the phenotype and clinical profile varies depending on the percentage of cells with the genome abnormality. Besides the characteristic 45,X karyotype in Turner syndrome, additional aberrations have also been reported. With a view to understand significance of additional chromosomal aberrations in Turner Syndrome, 424 referred cases were subjected to karyotypic analysis. Abnormal chromosome findings were seen in 42 (10%) cases, as follows: 45,X karyotype in 11 cases; 46,X,i(X)(q10) in 8 cases; 46,X,del(X)(q22) and 46,X,inv(X)(q23q28) in 1 case each. Mosaicism involving sex chromosomes was seen in 21 cases. Representative rarer cytogenetic findings in three cases with sex chromosome mosaicism with marker chromosomes are detailed. An 18-month-old female child with ambiguous genitalia, hernia, displaced gonads and enlarged uterus (Case-1). The other two patients included 12-year (Case-2) and 23 year old females (Case-3), clinically suspected to have Turner Syndrome. Conventional karyotyping – GTG banding, on 72 hour PHA stimulated peripheral blood cell cultures, using Cytovision 3.1 software, was the method of choice. As a confirmatory test and in an attempt to characterize the marker chromosomes, FISH for aneuploidy of chromosomes 13, 18, 21, X and Y was done using Vysis - Aneuvision kit and analyzed on Cytovision 2.7 software. Cytogenetic analysis by karyotyping showed 46,XY[44]/45,X[11]/46,X,+mar[5] in Case-1. Case-2 showed 45,X[44]/46,X,+mar[6] and Case 3 showed 45,X[37]/46,X,+r[4]/46,X,+mar[7]/47,X,+r,+mar[2] with ring structure distinctly observed in 6 metaphases. On FISH analysis, the origin of marker and the ring chromosome was observed as X chromosome. Thus, the study indicates presence of second X chromosome in

the marker and ring structure in the Turner Syndrome and not a complete loss of the X chromosome.

P 16

EXPERIENCE OF DISORDERS OF SEX DEVELOPMENT OVER FIFTEEN YEARS IN PGIMER, CHANDIGARH

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Introduction: Disorders of sex development (DSD) include a heterogeneous group of heritable disorders of sex determination and differentiation, formerly termed "intersexuality". The birth of a child with ambiguous genitalia still represents an enormous challenge. Unfortunately in our country many of the deliveries take place in the rural areas where sex assignment is not done by a doctor. This results in considerable delay in sex assignment causing psychological problems in the patient and family members.

Objective: To analyze the spectrum of chromosomal aberrations detected in patients presenting as ambiguous genitalia.

Methods: We performed a retrospective analysis of the last 15 years of all the cases referred to our laboratory with a diagnosis of ambiguous genitalia. The proforma of all the cases was evaluated for the clinical findings. In all the cases peripheral blood lymphocyte culture was done. The chromosomes were treated with trypsin and stained with geimsa. In cases where Y chromosome abnormalities were present, confirmation with Q banding was performed.

Results: In 15 years a total of 240 cases were referred for chromosomal studies for ambiguous genitalia. Of them 150 (62.5%) were children who were <12 years of age and 90 (37.5%) were adults. A total of 82 patients showed discrepant cytogenetics and clinical phenotype of them 44 were children and 38 were adults. In the 24 children with male phenotype, 21 were found to be 46 XY (genotypic females), one was female Turner (45 XO), one was mosaic Turner (46 XX; 45 XO) and one child had IsoXp. Nineteen phenotypically female children were detected to be genotypically male. One newborn with ambiguous genitalia was mosaic for 47 XXY. In the adults 3 individuals were brought up as males and had 46 XX and 28 individuals were brought up as females and had male genotype presented with primary amenorrhea and primary infertility.

Conclusions: Disorders of sex development present with various problems and the results show that there is considerable delay in identifying this condition possibly due to a high frequency of unsupervised deliveries being conducted by untrained staff specially in the villages. Special effort is necessary to optimize long-term surgical, medical, and psychological management.

P 17

EARLY DETECTION & THERAPY MONITORING IN METHYLMALONIC ACIDEMIA USING GAS CHROMATOGRAPHY / MASS SPECTROMETRY (GC/MS)

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Introduction: Methylmalonic acidemia (MMA) is a common organic acidopathy causing NICU & infant mortality & morbidity. Classical form is caused by complete deficiency of methylmalonyl-CoA mutase in neonates, presenting with severe metabolic acidosis, acute encephalopathy, hyperammonemia, & seizures. Late-onset mild variants are common responding to co-factor, vit. B12. Early detection is thus important to prevent developmental delay.

Objective: To conduct early & reliable diagnosis of MMA in high-risk cases by GC/MS, and check the efficacy of therapy by monitoring clinical & biochemical parameters.

Material: Total 2699 high-risk cases, > 90 % neonates and infants, were diagnosed for IEM by GC/MS using MILS method on urine sample. Among 844 (31%) abnormal cases, 8 % (67/844) were MMA. Pre and post therapy GC/MS analysis was done in 6 cases based on biochemical markers- methylmalonic acid, methylcitrate and tiglyglycine which were absent in the post treatment samples from the patients with clinical improvement.

Results: The incidence of MMA in high-risk cases was 1:40 with male: female ratio 1.4:1. Out of 6 cases, 3 were vit. B 12 responsive (50 %) as evidenced by the absence of marker compounds with neurological improvement. The remaining could be variants unresponsive to vit. B12 therapy.

Conclusion: All NICU babies need MMA screening. Early diagnosis with timely treatment contributes significantly to better prognosis of MMA patients. GC/MS analysis played an important role in checking the treatment response. Further mutational characterization will help the families in prenatal diagnosis & prevention in genetically diversified Indian population.

GENETIC SYNDROMES AND INTELLECTUAL DISABILITY – INDIAN SCENARIO.

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Introduction: The term 'genetic syndrome' refers to a recognized pattern of defects with specific etiology, and majority is with intellectual disability (ID). Pattern of multiple minor malformations and facial dysmorphism allows immediate clinical diagnosis of a genetic syndrome important in genetic counseling (GC) while explaining inheritance, recurrence risk and prenatal options.

Objectives: To diagnose genetic syndromes with ID (I.Q <70) at Referral Genetic Centre- CREMERE using clinical and chromosomal criteria, and frequency of common / rare syndromes in Indian Population.

Methods: The ID patients were medically examined by a genetic team. A detailed pedigree, medical history and dysmorphic features were noted with clinical photography. Karyotyping was done by high resolution G-banding method. Mutational study was performed in the few, viz Fragile- X syndrome, Rett syndrome and Cornelia de Lange syndrome.

Results: Of 1040 chromosomally analyzed ID patients, 142 were Down Syndrome (DS) indicating its high incidence (1 in 7). Other definite genetic syndromes identified from 242 cases were 51 viz Fragile- X (15), Rett syndrome (7), Turner syndrome (5), Angelman syndrome (5), Tuberous sclerosis (5) and Cornelia de Lange syndrome (5) and the other rare cases. Various syndromes, their incidence highlighting the importance of chromosomal and molecular diagnosis will be discussed.

Conclusion: Endogamous marriage and consanguinity make our population genetically vulnerable. Precise diagnosis of a syndrome by a genetic team is essential to guide the affected families. Screening for all pregnant women and carrier testing for chromosomal disorders will reduce the national burden of genetic disorders.

COMMUNITY GENETICS APPROACH TO POPULATING SCREENING IN INDIA FOR MENTAL RETARDATION- A MODEL FOR DEVELOPING COUNTRIES

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Introduction : Patient-doctor interactions, well established in routine health systems, vary with reference to the care of mentally retarded children in India and are more complex when mental retardation (MR) is due to genetic disorders / birth defects. Awareness about genetic disorders even among medical professionals is limited and incidence of MR in population is inadequate.

Objective: To screen some 0.55 million people living in semi-urban and slum populations for mental retardation by trained primary health centre (PHC) doctors, nurses and community health volunteers (CHVs) in Mumbai.

Methods: The staff was trained in the detection, prevention and diagnosis of MR, prenatal diagnosis, and reproductive responsibilities. Field visits were employed to confirm diagnosed developmental disabilities, and demographic data incorporating social maps of 14 PHCs were prepared. Cases detected by PHC staff with high-risk genetic factors were referred to CREMERE for cytogenetic and metabolic investigations, thus linking the study population and the Referral Centre. A genetic team interacted with the patient and family members for genetic counseling.

Results & Conclusion: Data confirmed MR in 511 of 525 detected, which otherwise would have gone unnoticed, reflecting the positive impact of training on the CHVs. Potentially preventable environmental factors, such as birth asphyxia, infections, and low birth weight were found in 251 cases (49%), 137 (27%) of which had additional genetic factors. Genetic causes were found in 186 (36%) individuals, the most common being Down syndrome. The study illustrates the urgent need for the integration of genetic screening into the public health services in India.

P 20

MORBIDITY AND HOSPITALISATION PROFILES OF PEOPLE WITH ANGELMAN SYNDROME AND PRADER-WILLI SYNDROME

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Introduction: Angelman (AS) and Prader-Willi syndromes (PWS) result from abnormal expression of imprinted genes at chromosome 15q11-q13. Disruption of the maternally-imprinted gene, *UBE3A*, causes AS, while loss of expression of paternally-imprinted gene/s leads to PWS.

Objectives: Data from Disability Services Commission files on people with AS and PWS were electronically linked with information from the state Genetic Services, Hospital Morbidity, and Mental Health datasets to create detailed profiles of clinical presentation, laboratory analyses, and hospital admissions.

Methods: Eighty patients with AS (19 female and 15 male; aged 6.5-39.0y), and PWS (23 female and 23 male; aged 0.9-48.3y) were included in the study. Age at diagnosis ranged from 0.1-27.0y, with younger mean ages in more recent patient cohorts.

Results: IQ scores of 25-54 were recorded for 31/34 individuals with AS, whereas 40/46 people with PWS had IQ scores of 40-70. The morbidity time-courses of the two disorders differed. Young children with PWS exhibited a range of health problems, including hypotonia and hypogonadism, requiring frequent hospitalisation, and the compulsive eating habits of older children and adults with PWS increased the probability of obesity and consequent clinical care. The comorbidities associated with AS were less acute, as indicated by comparatively fewer hospital admissions, but adolescents with AS were admitted more frequently than others in that age-group.

Conclusion: People with PWS were more likely to be admitted to hospital, and for longer periods, than either AS patients or the general population, a trend that was especially marked in the <5y and 25-34y groups.

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Unusual case of Neurofibromatosis Type 1 associated with unexplained intracranial bleed and Horse Shoe shaped kidney

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A 12 year old boy presented with sudden onset vomiting, headache and giddiness for 1 day. His mother and one of maternal uncle had NF 1. On examination vital were stable, blood pressure 110/70 mmHg. He had multiple café-au-lait spots, bilateral axillary freckles and Lisch

nodules in both eyes. Central nervous system examination revealed Glasgow Coma Score (GCS) of 14 with features of raised intra cranial pressure. CT scan of cranium showed intracranial bleed in right parietal region without midline shift. He was started on anti raised intra cranial pressure measures to which he responded gradually. Magnetic Resonance Imaging of brain showed an area of altered signal intensity involving right parietal region which was hypointense on T2 weighted image and isointense on T1 weighted image suggestive of hemorrhage. A T2 FLAIR hyperintensity focus was also seen in right globus pallidus and internal capsule. Magnetic Resonance Angiography (MRA) and Digital Subtraction Angiography (DSA) do not revealed evidence of Arterio-Venous Malformation (AVM) or aneurysm. Ultrasonography of abdomen revealed horse shoe shaped kidney which was confirmed by 99m Tc DMSA Renal cortical scan. There was no evidence of renal artery stenosis in Doppler study. Child is in follow up for last three months and is asymptomatic and with normal blood pressure records. The present case is unusual case of Neurofibromatosis Type 1 (NF1) since he had intracranial bleed in the absence of any other detectable cerebrovascular anomalies.

P 22

THE CONSANG-GOOGLE MAP DATABASE SYSTEM FOR THE STUDY OF CONSANGUINITY

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Introduction: As a working definition, marriages contracted between persons genetically related as second cousins or closer ($F \geq 0.0156$) are categorized as consanguineous. The ability to evaluate the incidence and types of consanguineous marriage in specific communities, regions or nations has become increasingly vital in terms of genetic education and genetic counseling programmes, and in assessing genetic association and genetic linkage studies.

Objectives: Using the website www.consang.net as starting-point, the aim was to create a user-friendly, interactive public database on the global prevalence of consanguineous marriage and its clinical, genetic and demographic outcomes.

Methods: A publicly accessible web application was constructed using data from www.consang.net with the open-source cakePHP framework (cakePHP.org) and Google Map Application Programming Interface (API) (maps.google.com).

Results: The resulting database provides an interactive and readily updatable global catalogue of consanguineous marriage, with precise geographical mapping of published studies. The visual display capacity has revealed important sampling and analysis patterns not previously resolved, in particular the unrepresentative regional clustering of data within specific countries.

Conclusion: Since its inception www.consang.net has proved to be a popular, frequently accessed website for both health professionals and the general public. The new Consang-Google Map will enable researchers to assess consanguineous marriage and its health outcomes with greater ease, clarity, and detail. It will also help in the planning and focus of future health-based studies on communities and areas for which current information is inadequate.

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GENETIC AWARENESS IN ALLIED HEALTH EDUCATION

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The Southern School of Natural Therapies, an accredited not-for-profit, private tertiary provider in Melbourne, Australia, offers degree programs in Naturopathy, Myotherapy and Chinese Medicine. These courses combine a basic degree in biomedical science with appropriate clinical instruction in four-year programs.

While the biomedical sciences are taught separately, considerable effort goes into imparting genetic awareness through knowledge of genetics, genetic diseases and genetic counseling in the middle years of the program prior to clinical instruction and supervision.

No preclinical subjects are more suited for imparting genetic awareness than in the social science subjects of Psychology, Counseling and Contemporary Health Studies. This is best illustrated in the use in counseling classes of a novel diagnostic and exploratory aid, devised at SSNT, called Genopedigrams (Storace, 2007)

Genopedigrams combine use of Genograms and Multiaxial Assessment as presented in the DSM-IV-TR (American Psychiatric Association, 2006), to illustrate the social dynamics and genetic links within families. It is a useful aid when developing treatment plans within a counseling context; it provides the Allied Health Practitioner with visual representation of the

social nature of family relationships as well as any genetic connection that may be influencing behavior and relations. Establishing familial genetic bases of particular behaviors informs the nature and course of subsequent therapy or referral.

The Allied Health Therapies are receptive to, and compatible with the aims of, genetic counseling, hence genetic awareness in allied health education cannot be overemphasized. Genopedigrams extend this awareness to embrace a holistic approach to patient management.

P 24

STUDY OF DERMATOGLYPHICS CHARACTERISTICS in DIABETES TYPE1 POPULATION IN IRAN

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Nowadays study of dermatoglyphics has a great importance in judicial and criminal researches. At present, study of dermatoglyphics that is related to some genetic diseases has an immense application. Relation of dermatoglyphics characteristics with some of diseases like Down's syndrome , Alzheimer's disease , multiple sclerosis , schizophrenia has been studied. Genetic factors are important in the causation of both types of diabetes.

Count of palm dermal ridges are very important in determining Quantitative fingerprint characteristics. Also dermal ridges in fingers have different shapes , similar loop, arch , whorl which are too important in Qualitative characteristics.

In this project we selected 30 patients and impregnated their palms with ink and pressed on papers and their results were compared with control group.

The results were analysed by statistical tests, i.e. T- test and chi-square.

The results indicated that a-b count in male and female patients has decreased comparatively to control group, but the reduction is not significant.

From the viewpoint of the shape of finger print, loop and whorl shapes are heterogenous and their number differ significantly comparative to control group. ($p=0.001, p=0.004$)

In summary, it seems that dermatoglyphics could be a suitable method for genetics studies and diabetes type1.

STANDARDIZING EBV VIRAL LOAD CUT- OFF IN HEALTHY, CONVALESCENT AND IMMUNOCOMPROMISED CHILDREN

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Introduction: EBV is a ubiquitous, pathogen that is harboured persistently by virtually all regardless of geographic location. Infection rates in young children are higher in developing countries and underprivileged societies. In India few studies regarding prevalence of EBV mainly by serologic and immunohistochemical markers are available. This ubiquitous virus can cause significant morbidity and mortality in the immunocompromised host such as renal transplant recipient .This study was undertaken to study detection of virus using nested PCR and real time approach in 20 normal and 40 convalescent children alongwith an index immunocompromised child with lymphoma..

Aim: To standardize DNA diagnostics of EBV in immunocompromised children by Real Time PCR.

Material & Methods: 20 normal children and 40 convalescent children recruited from Advanced Pediatric Centre (APC) PGIMER, Chandigarh. Nested PCR was carried out using two sets of primers and standard Raji cell line (EBV positive human Burkitt's cell line) as positive control. All samples were further analysed semi quantitatively by real time PCR using LC480 software.

Results & Discussion: A semi quantitative in-house test was devised. The fluorescence peak with 1/50 dilution of control DNA was used as marker against which other positive peaks were graded as low, medium and high positive. DNA of a 12 year old renal transplant recipient with clinically suspected EBV related B cell lymphoma was also tested by Real Time PCR. 5% normal and 20% convalescent children show positivity. However, the normal child showed a peak height almost similar to the child with lymphoma while 20% of convalescent children showed low levels of EBV viremia.

Conclusion: EBV infects normal as well as convalescent children. Normal children with intact immune response are able to resolve EBV infection with high viral load. On the other hand immunocompromised children can manifest EBV related lymphoproliferative disease with the same viral load. Thus EBV infection has to be correlated with immune status, clinical symptoms and viral load. Further studies with larger number of EBV related disease among

immunocompromised individuals will help to derive a diagnostic viral load cut-off in immunocompromised children.

P 26

GENETIC RELATIONSHIP AMONG FOUR DISTINCT POPULATIONS FROM INDIA BASED ON VNTR POLYMORPHISMS.

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Introduction: India is a country of enormous cultural, genetic and linguistic diversity. Several genetic marker studies have been conducted to study the genetic variations in different ethnic groups. The human genome contains tandemly repeated sequence elements. They show allelic variability in the number of repeat units; so represent a rich source of highly polymorphic markers useful for population diversity studies. We screened two VNTR markers (ApoB & D1 580) in four distinct population groups from India.

Objective:

- 1) To study allele frequencies of two VNTR loci (ApoB & D1S80) in Lohanas, Vatalia, Prajapatis, Parsis & Oraons.
- 2) To look for genetic relationship amongst these groups on the basis of two VNTR markers.

Methods: Blood samples from unrelated individuals from the above mentioned groups were collected. Sample size ranged between 55-115. Genomic DNA was extracted using a standard protocol. The ApoB and D1S80 locus analysis was done by Amplified Fragment Length Polymorphisms (AFLP). Statistical analysis was performed to look for allele frequencies and genetic relationship.

Results: In ApoB, the 631 bp allele (33 repeats) was the most common alleles in all the four groups. (.3155-.5370). One rare allele; 361 bp (15 repeats) was seen in all populations except Oraons. Totally, 9-11 alleles were seen in these four populations.

In D1S80, 531 bp (24 repeat) and 435 bp (18 repeats) alleles were the most common alleles. However, among the Parsis the 515 bp (23 repeats) allele was the most common allele. Totally 13-18 alleles were seen in the populations.

Conclusion:

- 1) Both these markers are highly polymorphic in these four populations.

- 2) Based on these two loci, Vatalia Prajapatis and Lohanas formed one cluster while Parsis and Oraons remained as distinct groups.

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GENETIC SCREENING OF BOVINE LEUKOCYTE ADHESION DEFICIENCY (BLAD)

IN PAKISTAN

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Bovine Leukocyte Adhesion Deficiency (BLAD) is a lethal immunodeficiency disease. Its mode of inheritance is autosomal recessive, Caused by the inability of leukocytes to migrate to sites of infection and tissue injury to eliminate invasive microorganisms in an immune response. This ability of migration of leukocytes is dependent on the coordination of the adhesion molecules on their surfaces and on endothelial cells. These molecules include integrins. β_2 integrins are exclusively expressed on leukocytes. The molecular basis of BLAD is a single point mutation A-G in the gene encoding the β_2 (CD18), cause D128G substitution in glycoprotein near the center of 26 consecutive amino acids that are identical in normal bovine, human and murine. This condition is found in the Holstein-Friesian population and has originated in the USA, from where it spread throughout the world, mostly by Artificial Insemination. Present study was planned to standardize a technique for the diagnosis of the BLAD and to get a first report on the presence of BLAD Allele in Pakistani cattle population. Whole blood was collected from 600 animals for this study, Holstein Friesian (HF), Friesian Sahiwal (FS) and Sahiwal cows were sampled from Livestock Experiment Station (LES) Qadirabad and Private farms. Similarly Sahiwal, HF and FS bulls present at Semen Production Unit (SPU) Qadirabad & SPU Kherimorat Attock as candidate bulls in progeny testing programme were sampled. The identification of normal, carrier and affected animals for CD18 locus causing BLAD was made by PCR-RFLP method the results were confirmed through sequencing. Genetic frequency of mutant allele in HF and FS population in Pakistan was calculated as 0.02.

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EFFECT OF METHANOLIC EXTRACT OF ALLIUM SATIVUM (AS) IN DELAYING CATARACT IN STZ-INDUCED DIABETIC RATS

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Glycemic induced stress is a major culprit contributing to oxidative insult that has far reaching effects in diabetic cataract worldwide. In an attempt to prevent/delay cataract, many therapeutic agents have been identified and among these, natural dietary sources have gained pharmacological significance. Hence, we investigated the efficacy of the methanolic-garlic extract against diabetic cataract in wistar rats. Methanolic garlic extract scavenged the transition-metal ion generated H_2O_2 with an IC_{50} of $768.8 \pm 1.76 \mu g/ml$, showing its potential ability as an antioxidant. We have noticed lenticular opacity and oxidative damage in streptozotocin (STZ) induced hyperglycemic rats. This is evident by the elevation of Ca^{2+} , Cu^{2+} , Na^+ , Mg^{2+} , Thiobarbituric acid reacting substances (TBARS), carbonyl content and increased activities of polyol enzymes, glutathione peroxidase (GPx), Superoxide dismutase (SOD) and up-regulation of iNOS transcript and protein aggregation/ cross linking followed by a decrease in Glutathione (GSH), K^+ content and tryptophan fluorescence in the cataractous lenses of STZ induced diabetic rats. Garlic administration in a dose-dependent manner attenuated the glycemia mediated oxidative stress as all the parameters have been found normalized more or less to that of control rats and thus delaying the progression of the lens opacity. We hypothesize that garlic-extract has hypoglycemic and anti-oxidant properties that can delay the progression of cataract as revealed in this study.

P 29

ELEVATED EXPRESSION OF INDOLEAMINE 2, 3-DIOXYGENASE (IDO) AND ACCUMULATION OF KYNURENIC ACID IN THE PATHOGENESIS OF STZ-INDUCED DIABETIC CATARACT IN WISTAR RATS

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Introduction: Indoleamine 2, 3-dioxygenase (IDO) is an interferon (IFN)- γ -inducible, enzyme that catalyses the initial and rate-limiting step in the degradation of the essential amino acid tryptophan via. the kynurenine pathway. Fluctuations in the levels of kynurenines have been implicated in the pathogenesis of cataract. The aim of the present study was to determine the

expression and activity of IDO, and the levels of tryptophan (TRP) and kynurenic acid (KYNA) in the streptozotocin (STZ)-induced diabetic cataract in Wistar rats.

Methods: Male Wistar-NIN rats were divided into two groups: (a) non-diabetic control (G-I), and (b) diabetic (G-II) and maintained for 8 weeks on AIN-93 diet. Diabetes was induced by a intraperitoneal injection of STZ (34 mg/kg body weight). A slit lamp biomicroscope was used to monitor the progression of cataract induced by hyperglycemia. The rats were sacrificed at the end of 8 weeks, and the levels of key enzymes in several biochemical pathways (kynurenines, polyol, oxidative stress, and redox systems) that play a critical role in the pathogenesis of cataract were determined.

Results: Mature cataract was observed 8 weeks after the injection of STZ in G-II rats. The levels of mRNA and enzyme activity of IDO, as well as the content of tryptophan (TRP) and kynurenic acid (KYNA) were elevated in these rats. In addition, levels of polyol enzymes and oxidative stress markers were significantly increased along with alterations in antioxidant reserves.

Conclusions: Our observations indicate a correlation between the levels of tryptophan (TRP), kynurenic acid (KYNA) and polyol enzymes with the formation and progression of diabetic cataract in Wistar rats.