

## BASIC MEDICAL SCIENCES

As in the previous years the major activities of the Council in basic medical sciences have been carried out in the fields of biochemistry, cellular and molecular biology, genomics, haematology, genetics, immunology, pharmacology, toxicology and traditional medicine. Intramural research in the fields of pathology and haematology is being carried out at Institute of Pathology (IOP), New Delhi and Institute of Immunohaematology (IIH), Mumbai respectively.

### PATHOLOGY

#### TUMOUR BIOLOGY

##### Breast Cancer

##### Study of Candidate Genes Associated with Breast Cancer Susceptibility

Study is aimed to identify low penetrance genes associated with breast cancer risk in north Indian population. Polymorphisms/mutations in low

penetrance genes, CYP17, VDR and GST were analyzed in breast cancer patients and in controls. Screening for A2 polymorphism in CYP17 gene increased incidence of heterozygous and homozygous carrier of A2 polymorphism in early onset breast cancer cases which is responsible for extra site for estrogen synthesis and higher circulating estrogen levels.

Studies have also been initiated to investigate the role of low penetrance VDR gene polymorphism(s) for giving susceptibility to familial and sporadic breast cancer. It was investigated whether 3' UTR (Taq I, Apa I) polymorphisms in the VDR gene have any association with altered breast cancer risk in north Indian population.

A series of 204 breast cancer patients along with 200 controls were screened for Apa I (intron 8) and TaqI (exon 9) polymorphisms in VDR (Fig. 1) gene. Patients included 105 early onset, 65 late onset and 34 familial cases. No statistically significant association was found between polymorphic VDR genotype with breast cancer risk. Further, Poly A repeat in VDR gene will be studied to find the genetic linkage with ApaI/TaqI cluster in the 3'UTR region which is important for regulation of mRNA stability.

Besides this GSTs were also studied in the same population. *GSTM1* genotype was analyzed in 80 breast cancer patients and 17 controls. Null genotype was observed in 27% patients (13.7% early onset and 12.5% late onset type) while 73% showed wild type alleles. Among controls null genotype was observed in 29% and wild type allele in 71% individuals. Cases and controls were also analyzed for *GSTT1* genotype. Null genotype was found in 37% patients and wild type in 63% cases. *GSTT1* null genotype was

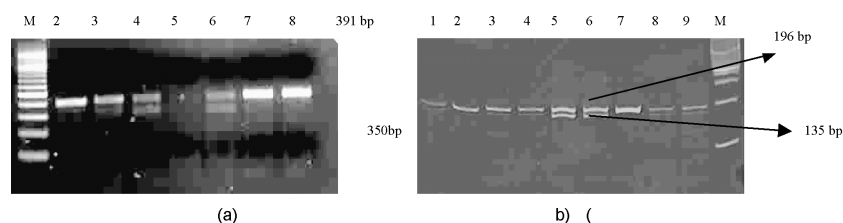


Fig. 1. Ethidium bromide-stained gel showing (a) *TaqI* digested PCR product of VDR gene. Lane 1 contains m.w. marker (100-bp ladder). Lanes 2,7 and 8 show intact amplicon of 391 bp. Lanes 3,4 and 6 show amplicons of 391, 350 and 41 bp, representing heterozygous pattern (b) *ApaI* digested PCR product showing amplicons of 196, 135 and 61 bp. Lanes 1, 2, 3, 4, 7, 8, 9 show intact amplicon. Lanes 5 & 6 represents heterozygous pattern.

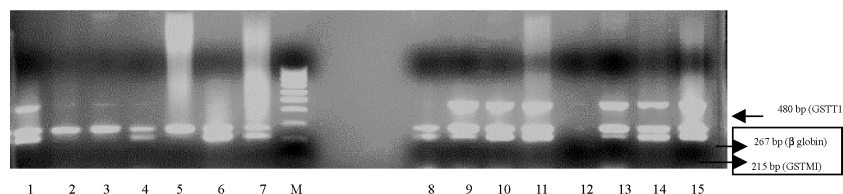


Fig. 2. Genetic alterations in *GSTM1* and *GSTT1* in breast cancer patients. Presence of 480 bp PCR product shows presence of wild type *GSTM1* gene, 215 bp PCR product shows presence of wild type *GSTT1* gene.  $\beta$ -Globin gene was used as internal control which was detected by the presence of 267 bp PCR product. Lanes 2,3,4,5,6,7 & 8 show *GSTT1* null mutation and lanes 2,3 & 5 show *GSTM1* null mutation

observed in 29% and wild type allele in 71% control individuals in 35% early onset and 40% late onset patients (Fig.2).

### Study of Molecular Characteristics of Familial and Early Breast Cancer

Studies on molecular characterization of early onset and familial cancer have shown decreased expression of estrogen receptors (ER) in early onset and familial cases and increased expression of cyclin D in familial cases. Study was undertaken to characterize molecular changes in oncogenes, tumour suppressor genes, apoptotic genes and DNA mismatch repair genes of early onset breast cancer with or without family history and mutations in BRCA 1 /2 genes.

The cytokeratin (CK 5,6,7 and 14) profile was studied in 57 cases of breast cancer to evaluate the cellular origin, (*viz.* basal or luminal cells) and for functional classification of breast cancer. Immunohistochemical profile of CK5/6 and CK7 was studied in paraffin embedded sections. The basal cytokeratin CK5/6 was expressed in 26.3% and luminal cytokeratin CK7 in 8.69% cases (Fig.3). CK14, studied in 30 cases was seen to be expressed in 20% cases. Correlation of these cytokeratins with molecular profile of breast cancer cells in different groups is being studied.

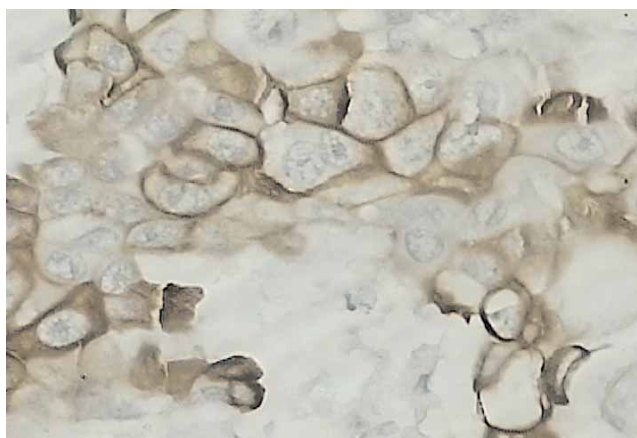


Fig. 3. Cytokeratin 7 expression in a case of infiltrating ductal carcinoma

Androgen receptor (AR) positivity was observed in 40% carcinoma breast patients (Fig.4). ER immunostaining was seen in 35% cases while 44% cases were positive for progesterone receptor (PR)

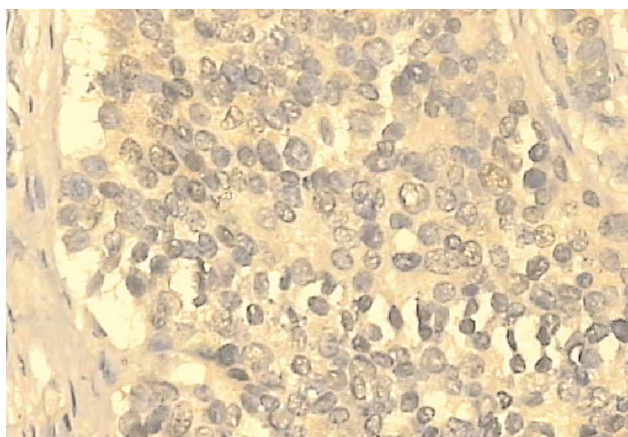


Fig. 4. Androgen receptor nuclear immunopositivity in infiltrating duct carcinoma of breast

expression. Immunostaining for EGFR was positive in 40% cases while TGF-beta receptor showed positivity in 32% cases. Statistically significant higher positivity for ER, PR and TGF-beta receptors was observed in cases positive for AR. EGFR positivity also showed a higher trend in AR positive cases but was not statistically significant. Of the total 30 familial cases of breast cancer, ER and PR expression was seen in 33.3% and 56.6% respectively. AR positivity was found in 50% while EGFR was expressed in 46.6% of familial breast cancer patients. TGF-beta receptor positivity was seen in 33.3% cases.

### Urogenital Malignancies

#### Study of the Host Immune Response in Patients with Superficial Transitional Cell Carcinoma

The study evaluated the cytokine (Th1 and Th2) balance in peripheral blood mononuclear cells (PBMCs) of transitional cell carcinoma (TCC) patients in order to assess the immunological factors influencing the anti-neoplastic activity of intravesical combination BCG/IFN  $\alpha 2\beta$  in patients. Study was done to measure the dynamics of systemic Th1 and Th2 cytokine responses in patients who had not received any therapy prior to transurethral resection of bladder tumour (TURBT) (pre-therapy) and the immune responses in the same group of patients were compared following TURBT and intravesical combination therapy (post-therapy) using flow cytometry.

Forty-one patients with histologically confirmed superficial TCC treated by TURBT followed by

intravesical combination immunotherapy formed the study group. Patients were followed up every third month for duration of 6 months to 3 years (median follow up of 22.5 months). The circulating levels of Th1 and Th2 cytokines were determined in the sera of normal healthy subjects (20) and superficial TCC patients prior to therapy (41, pre-therapy) and following combination BCG/IFN  $\alpha 2\beta$  intravesical therapy (37, post-therapy). The mean levels of Th1 cytokines, IL-2 and TNF $\alpha$  were significantly reduced whereas significantly enhanced levels of Th2 cytokines like IL-4, IL-6 and IL-10 were observed in pre-therapy patients as compared to healthy volunteers (Fig.5). The mean levels of IFN  $\gamma$ , IL-2 and TNF $\alpha$  increased whereas the Th2 cytokines, IL-4 and IL-10 were concomitantly reduced in patients following combination treatment as compared to patients prior to intravesical treatment (Fig.6).

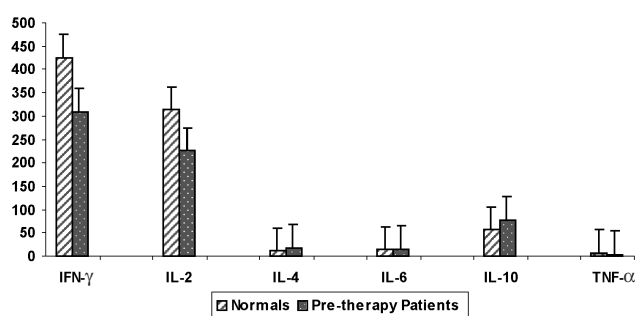


Fig. 5. Th1/Th2 cytokines in normal and pretherapy patients

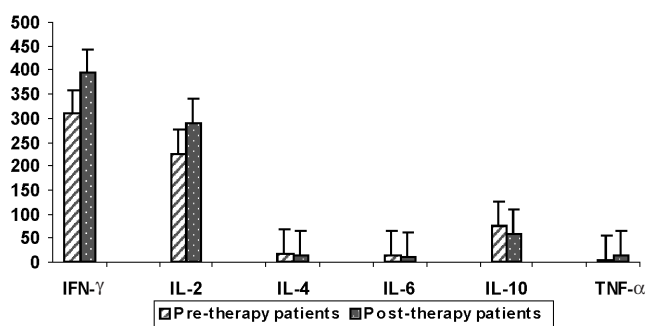


Fig. 6. Expression of Th1/Th2 cytokines in TCC patients prior to and following intravesical combination immunotherapy

The expression of p53, p21<sup>waf1</sup> and growth factor receptors such as VEGFR and TGF $\beta$ R was

studied in 32 cases of superficial TCC to evaluate the relevance of transforming proteins in the progression of superficial bladder cancer. Expression of p53 was seen in 68.75% cases, and that of VEGFR in 46.87%. Loss of p21<sup>waf1</sup> was observed in 32.75% cases and TGF $\beta$ R in 56.25% cases. The study showed that while p53 and VEGFR expression was increasing with increasing grade of tumour (Fig.7), TGF $\beta$ R decreased with increasing grade of tumour (Fig.8). The study also showed that the biomarker profile of p53+/VEGFR+/p21+/TGF $\beta$ R – was a predictor of recurrence, progression and decreased survival.

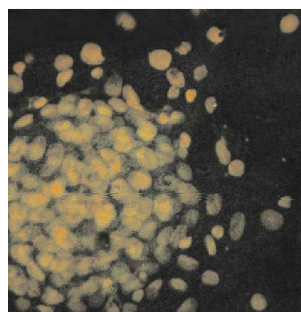


Fig. 7. VEGFR expression in a case of TCC bladder on cell spots

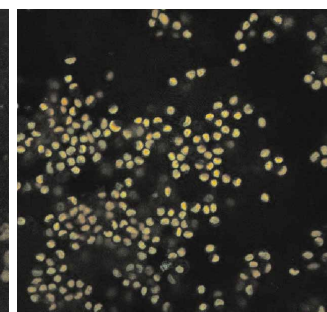


Fig. 8. TGF $\beta$ R expression in TCC bladder

## Hematopoietic-Lymphoid Malignancies

### Prognosis and Response to Chemotherapy in Acute Leukaemia Patients

Earlier studies revealed that pattern of *in vitro* sensitivity of leukaemic cells to chemotherapeutic drugs vincristin, daunorubicin, methotrexate, cytosine arabinoside and L-asparaginase can be correlated with clinical response of the patients. During the year expression of genes commonly involved in drug resistance was studied by real-time RT-PCR (TaqMan probe assay) to see if gene expression could be indicator of drug sensitivity pattern in individual patient samples. The mean mRNA expression level of the selected genes (MDR, DHFR, GST-pi, p53) was calculated for each case. Of the 12 samples analyzed for the relative expression of genes DHFR, GST, MDR and p53, 6 samples were of acute myeloid leukaemia (AML), 5 of acute lymphoblastic leukaemia (ALL) (3 of B-ALL, 2 of T-ALL) and 1 of biphenotypic leukaemia.

Preliminary results showed mean GST-pi expression significantly upregulated in AML and T-ALL, mean MDR expression significantly upregulated in AML and downregulated in B-ALL, T-ALL and biphenotypic leukaemia, mean DHFR expression significantly upregulated in T-ALL as well as in AML and B-ALL and mean p53 expression significantly upregulated in AML and T-ALL (Fig.9). More samples are being analyzed to see if chemosensitivity can be correlated with expression pattern of these selected genes.

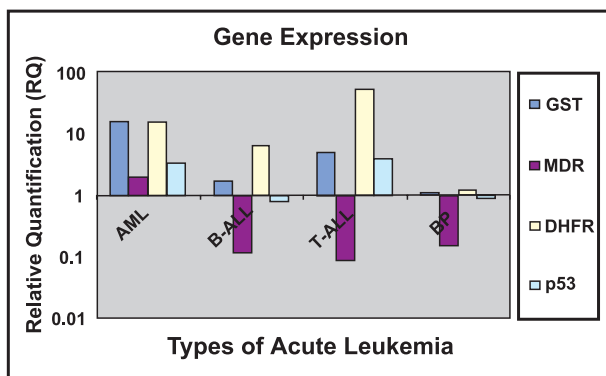


Fig. 9. Gene expression profile of GST-pi, MDR, DHFR, and p53 in leukemic samples

### Expression of Cell Cycle Regulators in Acute and Chronic Myeloid Disorders

Chronic myeloproliferative disorders remain stable for years and often transform to an accelerated phase of blast crisis. Study was done to understand the significance of expression of cell cycle regulators (p53, p21 and MDM2) and proliferative and apoptotic markers (PCNA and CPP32) in bone marrow specimens of 40 patients [15 with AML, 15 with chronic myeloid leukaemia (CML) and 10 with myelodysplastic syndrome (MDS)]. p53 expression was found in 28%, p21 in 25% and MDM2 in 23% samples. p53 expression was higher in AML while p21 and MDM2 expression was higher in CML. PCNA and CPP expression was highest in MDS. Twenty per cent samples showed overexpression of P21 and MDM-2 in the absence of p53 expression indicating that p53 independent pathway may be involved in cell cycle regulation in these samples. MDM-2 expression was highest in CML with blast crisis and was significantly associated with low/absent apoptosis suggesting that MDM2 overexpression

may be related to a higher grade of myeloid proliferative diseases and its progression to acute leukaemia.

## PATHOLOGY OF INFECTIOUS DISEASES

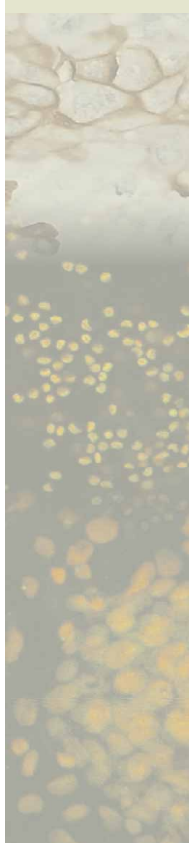
### Genital Chlamydia

#### Development of Diagnostic Assay for *C. trachomatis*

During the year under report, determination of sensitivity and specificity of developed antichlamydial monoclonal antibody clones was done in addition to their characterization by Western blot. Sensitivity of clones was determined by monoclonal antibody titers against *C. trachomatis* antigen by immunofluorescence assay. Further the reactivity of three developed antichlamydial clones (D10.4, H.5.6 & B2.2) was evaluated in 200 endocervical specimens by enzyme immunoassay (EIA) and compared with cell culture. The positivity for clones in EIA was 45%, 43% and 35% respectively when compared with cell culture method which was 46%. The maximum positivity was detected with species specific clone (D10.4) which can react with any serovar/ serovars of *C. trachomatis* followed by clone (H.5.6) D serovar specific, thereby indicating a high prevalence of serovar D of *C. trachomatis* in female genital tract.

#### Cytokine Gene Expression Profile in Endocervical Lavages in *C. trachomatis* Infected Women

Cell mediated immune responses play a major role in clearance and resolution of chlamydial infection. Further, chlamydial infection at mucosal sites provides sustained antigenic stimuli for host immune responses with the induction of cytokine production. Cytokine production profiles of PBMC and lavage cells in response to stimulation with *C. trachomatis* antigen and PHA were compared in patients positive for *C. trachomatis* infection and in controls. Levels of IFN  $\gamma$  and IL-10 were found to be higher in cervical lavage cells of positive cases when compared with controls which suggests a mixed type of immune response and a probable establishment of conditions within the host cell milieu for long term persistence of *C. trachomatis*.



### Role of Chlamydial Heat Shock Proteins (cHSP) in Pathogenesis of Genital Tract Infection in Women

cHSPs are known to be immunopathogenic proteins due to their structure homology with host HSP proteins, which contribute to persistence of *Chlamydia* inside the host cell. Responses to cHSP60, have been associated with sequelae of upper genital tract disease in infected women. Study was done for cloning and expression of *chsp60* gene in order to elucidate its pathogenic role and further development of diagnostic kit for detection of *Chlamydia* infection by serological diagnosis of chlamydial heat shock proteins 60 and 10 (cHSP60 & cHSP10) by ELISA using specific peptides. cHSP60 and cHSP10 showed strong positivity in *C. trachomatis* positive patients compared to negative patients or patients positive for other sexually transmitted pathogens. The sensitivity and specificity of cHSP60 in comparison to DFA was 76.9% and 89.3% respectively.

### Inclusion Protein and Their Contribution towards Chlamydial Pathogens

During developmental cycle, the intracellular bacterial pathogen *C. trachomatis* remains confined within a vacuole known as an inclusion. It is thought that pathogen-derived products are transported through this membrane, where they combine with host cell factors necessary for bacterial development. Despite the complete genomic sequencing of *C. trachomatis*, the identity and function of most *Chlamydia* gene products that interface with the host cell remain a mystery. At a molecular level the inclusion membrane (Inc) defines the critical interface between *Chlamydia* and the host cell.

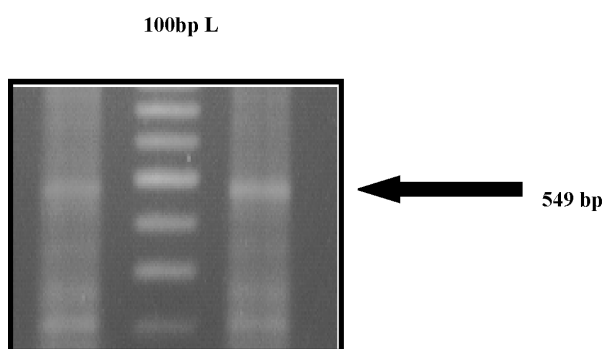


Fig. 10. Agarose gel electrophoresis for amplified product of Inc gene showing 549 bp band.

Therefore, study was done for cloning and expression of Inc gene which may be responsible either for the protective/pathogenic function or in the development of *C. trachomatis*. For this study, primers were designed for inclusion gene and PCR was standardized. Optimum condition for amplification of inclusion gene was achieved and a PCR product of 549bp was obtained (Fig.10). Further cloning and protein expression is in progress.

### Role of Iron in Pathogenesis of *C. trachomatis* Infection

Iron deprivation synergistically helps *C. trachomatis* to decrease levels of reactive oxygen species (ROS) in late stage of development thereby helping chlamydiae to persist. To study the role of iron in persistence of chlamydial infection, levels of ROS *in vitro* in culture system in different conditions were evaluated. It was found that during *Chlamydia* infection, there was decrease in ROS levels (75 to 35) which further decreased on addition of iron chelator (dichlorofluorescein, DCF) (Fig.11). In addition, studies were targeted on the transferrin receptor mediated iron uptake, which may lead to ROS generation resulting in host apoptotic cell death. Higher expression of Bcl 2 (anti-apoptotic protein) was detected in *Chlamydia* infected cells.

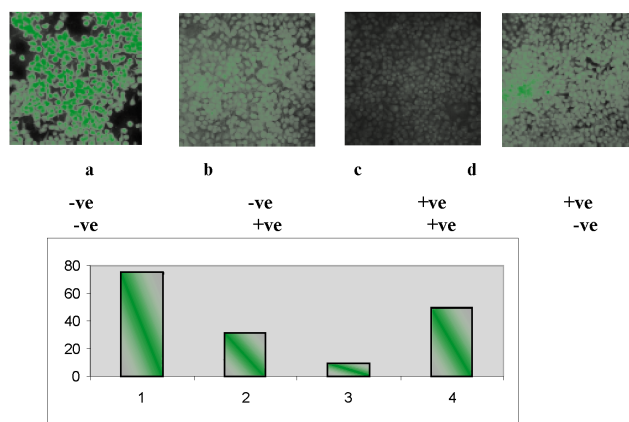


Fig.11. Level of reactive oxygen species (ROS) as probed through dichlorofluorescein (DCF) in different conditions at 48 hpi of *C. trachomatis* and visualized by confocal microscopy a) Culture of McCoy cells alone b) McCoy cell culture -ve for *C. trachomatis* where DCF was added c) McCoy cell culture infected with *C. trachomatis* + DCF added d) culture CT infected but DCF negative

### Immune Responses to *C. trachomatis* Infection in Spontaneous Aborters

Study was undertaken to find the precise immunological mechanism whereby infection with *C. trachomatis* adversely affects early stage pregnancy in women leading to miscarriage. Spontaneous aborters (SA) in the I<sup>st</sup>/II<sup>nd</sup> trimesters of pregnancy were enrolled alongwith their male partners and samples of non-macerated aborted endometrial curettage tissue (ECT) and blood were collected. After ruling out infection with other pathogens in SA and presence of male factors in their partners, SA were divided into group I, *i.e.* those with no past history of spontaneous abortion and group II, *i.e.* those with past history of  $\geq 1$  spontaneous abortion. The prevalence of chlamydia in group I was 16.6% and 10.7% by IHC and PCR respectively in ECT while in group II it was 12.5% by IHC/PCR. Cytokine expression in ECT is being studied in CTR infected SA.

### Leishmaniasis

#### Discovery of Virulence-related Genes in *Leishmania* using Microarray

Genomic microarray based analysis was carried out using various stages of the parasite during transformation from promastigote to amastigote stage. To understand the process of differentiation of *Leishmania*, global variation in gene expression in promastigotes, an intermediate stage of differentiation (PA24) and axenic amastigotes in culture was assessed using a *L. donovani* genomic microarray with 4224 clones in triplicate. During intermediate stage of differentiation 24h after shifting the promastigotes into amastigotes (PA24), there were 68 (~1.5%) clones with  $\geq 1.7$  fold higher expression in comparison with promastigotes, whereas in terminally differentiated amastigotes there were 239 (~5.65%) such clones. Of particular interest were certain genes that exhibited a transient increase or decrease in expression at the PA24 stage. Kinases showed a transient increase and surface molecules PSA and amino acid permeases showed a brief decrease at the PA24 stage. Also, the proportion of differentially expressed genes that represent surface molecules was greater in Am/Pro microarrays than in PA24/Pro microarrays. The microarray results have

been validated using Northern blots or RT-PCR. The results provide important leads about the genes involved in the early differentiation process of *L. donovani* that may contribute to virulence.

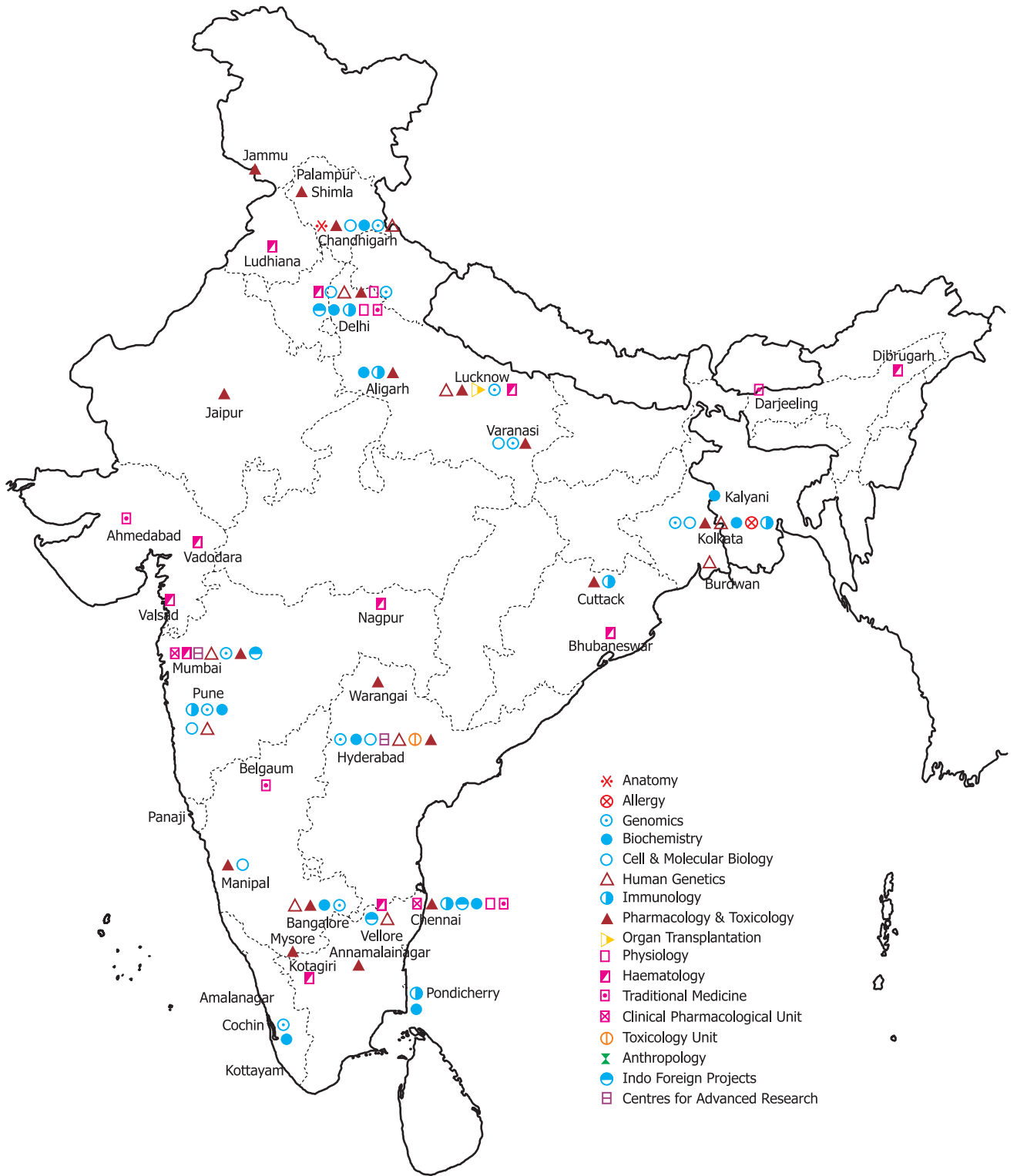
#### Evaluation of Localized Immune Response in PKDL Patients

Localized responses in KA and PKDL were studied using semi-quantitative RT-PCR. Intralesional cytokine gene expression was analyzed in 28 PKDL and 14 KA patients. Data revealed mixed Th1 and Th2 responses, as reflected by elevated IFN- $\gamma$ , TNF- $\alpha$ , TGF- $\beta$ , interleukin (IL)-10, IL-6 and IL-4 with minimal expression of IFN- $\gamma$ R1 message in PKDL lesions compared to normal skin tissue. In comparison with KA, message for IFN- $\gamma$ , TNF- $\alpha$  and IL-6 were found significantly elevated in PKDL lesions, implying an important role of these cytokines in PKDL pathogenesis.

#### Identification and Characterization of *L. tropica* Isolated from Indian Patients of Cutaneous Leishmaniasis

Study was carried out for molecular and immunological characterization of *L. tropica* species causing cutaneous leishmaniasis (CL) in Bikaner, Rajasthan. Clinico-epidemiological analysis of 98 cases suggested that the preponderance of infection is higher in males than in females with highest prevalence in the age group of 5 to 30 yr and variability in lesion types and number. Comprehensive molecular and immunological studies were carried out for identification and characterization of species in 32 cases. Culture was positive in 43.75% cases and direct microscopy in 59.3% cases. To affirm if the clinical isolates belonged to *L. tropica* species, isolates were subjected to nested ITS-1 PCR-RFLP analysis. Result yielded typical band patterns of *L. tropica* in DNA from both culture and clinical samples. That isolates belonged to *L. tropica* was confirmed by IFA using species-specific monoclonal antibodies. Comparative analysis showed that kDNA minicircle PCR primers were 84.37% sensitive for detecting the parasite directly in clinical samples. The present study is the first





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comprehensive molecular and immunological investigation of CL caused by *L. tropica* in India which carries immense importance from epidemiological and treatment point of view.

## Other Studies

### Use of Placental Tissue in Human Environmental Bio-monitoring of Pollutants

During the year under report, approximately 4000 pregnant women attending Safdarjung Hospital OPD were screened for possible exposure to pollutants. A total of 150 women who reported exposure to agricultural chemicals during pregnancy and 100 placental samples collected from them at the time of delivery were selected. Pesticide residues (synthetic pyrethrins) and pollutants (like cycloheptatrienylium bromide and naphthalene) were detected from placental extracts.

## BIOCHEMISTRY

Studies on the effect of chronic ethanol feeding on transport of folate and expression of its transporter in rat intestine were done at PGIMER, Chandigarh. Folate deficiency is the most common sign of chronic alcoholism. Intestinal malabsorption of the folate is a contributing factor of alcohol induced folate deficiency in male Wistar rats fed 1g/kg body weight ethanol orally for 12 weeks. Chronic ethanol feeding was found to decrease the binding as well as transport of folic acid by altering various kinetic characteristics of the processes at both the intestinal and renal brush border membrane level. Decreased uptake of folate across the intestinal epithelium was associated with reduced expression of mRNA of folate carrier. Moreover, transport was reduced all across the crypt villus axis after chronic ethanol feeding. Using various activators and inhibitors of different signaling pathways it was observed that folate transport is regulated by cAMP dependent protein kinase A.

A study on antioxidant vitamin supplements as palliative treatment of bone disorders was done at Dr. D.Y.Patil Medical College, Pune. A group of 198 patients suffering from skeletal disorders like osteoporosis, renal osteodystrophy, bone malignancy and fractures underwent baseline assessment of biochemical markers viz. [osteoblastic markers: serum alkaline phosphatase

(ALP), free Ca<sup>++</sup> and inorganic phosphorus (Pi); osteoclastic markers: serum tartarate resistant acid phosphates (TrACP) and malon dialdehyde (MDA) and the antioxidant markers: serum superoxide dismutase (SOD) and erythrocyte reduced glutathione (GSH)]. The test group was then divided into 3 groups—group A (Evinal 400mg), B (Celin 500mg) and C (Evinal + Celin) for antioxidant supplementation for a period of 90 days. There was significant improvement in levels of serum MDA, serum TrACP, serum ALP, SOD and GSH. The response improved with duration of supplementation. Results revealed that antioxidant vitamin E and C individually or conjointly improve the bone status in various skeletal pathologies and hence may serve as cost effective, palliative supplements in addition to curative treatment.

## IMMUNOLOGY

A study on antigen-dependent lymphocyte activation thresholds in adaptive immunity aimed at examining the effects of antigen-induced structural changes in antigen receptors on biochemical control of lymphocyte activation was done at International Centre for Genetic Engineering and Biotechnology and National Institute of Immunology, New Delhi. Studies revealed for the first time the relationship between signal amplitude and its duration during signal transduction. Study on B cells demonstrated that amplitude of the initial pulse of the signal dictates how far and how fast it will travel through the downstream networks. Further, the amplitude of the initial pulse was shown to be dependent upon an amplification system that was controlled through a feed back loop between cytoplasmic calcium ions and reactive oxygen.

## GENOMICS AND MOLECULAR MEDICINE

Studies to understand the mechanism of transcription termination in mycobacteria were done at IISc, Bangalore. Intrinsic transcription termination signals provide an extremely economical mechanism for the termination process since they can function in the absence of proteins. So far the process of termination has been mainly studied in *E. coli* and confined to *E. coli* paradigm. A versatile algorithm

(GeSTer) for genome-wide analysis of transcription termination signals has been developed. The new algorithm is applicable for the analysis of potential terminators from all sequenced bacterial genomes. In addition to the classical *E. coli* (or text book) type terminators, the algorithm identifies several new kinds of structures as putative terminators. By *in vitro* and *in vivo* experiments, these novel structures were shown to function as effective terminators in mycobacteria. The different kinds of terminators are concentrated within the first 50 nucleotides downstream of coding sequences in most bacterial genomes indicating that they serve the same purpose. The algorithm is fastest and most accurate for identifying the terminators and other secondary structure motifs involved in regulation of gene expression. In addition, it would serve as an invaluable tool in predicting the organization of genes, operons and for the development of expression systems.

Study for molecular diagnosis of common opportunistic infections in cancer was completed at Kidwai Memorial Institute of Oncology, Bangalore. Frequency and type of opportunistic infections (invasive fungal infections (IFI), *T.gondii*, *P.jirovecii* and cytomegalovirus infections) in various types of malignancies were determined using PCR. The cause of fever due to the above mentioned pathogens was investigated by molecular and conventional means in a total of 200 episodes of fever (182 with haematological malignancies and 18 with solid tumours; 132 with febrile neutropenia and 68 with fever but with normal counts). Fever could be ascribed to IFI in 16% cases *T.gondii* in 13.5%, *P.jirovecii* in 11% and CMV in 5.5% cases.

Molecular genetic analysis of some forms of inherited childhood blindness and genetic counselling of affected families was done at L V Prasad Eye Institute, Hyderabad. The study involved molecular genetic characterization of 3 ocular diseases affecting children—retinoblastoma, primary congenital glaucoma (PCG) and anterior segment dysgenesis. Retinoblastoma is a childhood intraocular malignancy resulting from mutation of both alleles of the R81 gene. Standardization and screening of 50 retinoblastoma patients for mutations was carried out by SSCP. Mutations detected included a few novel as well as recurrent mutations. Majority of mutations result in premature termination of protein.

PCG is a form of glaucoma manifesting at birth. This study revealed mutation of CYP181 causing PCG in Indian populations. The R368H mutation was found to be the most prevalent CYP181 mutation. In addition, it was found that the myocilin gene is a cause of PCG in some families. Anterior segment dysgenesis is a group of disorders arising from developmental anomalies in the anterior segment of the eye. The PAX6 gene for aniridia and the FOXC1 gene for Axenfeld Reiger anomaly were studied and found to have novel mutations in the patients tested. Overall, the results of the study provide data on these disorders in Indian patient populations for the first time and can be useful in devising screening/genetic counseling strategies for patients.

## HUMAN GENETICS

A study was conducted at Indian Statistical Institute, Kolkata on the effect of Alu insertion/deletion polymorphism at the DCPI locus on blood pressure and lipid levels among individuals belonging to defined haplogroups from two tribal populations—Toto and Bhutia of India. Toto are exclusively rural while Bhutias live in both rural and urban habitats and their dietary habits and lifestyles have also changed in recent decades. About 283 samples from Totos and 455 from Bhutia were obtained. The individuals were also genotyped with respect to insertion/deletion polymorphism at the DCPI (ACE) locus to estimate the impact of this polymorphism on blood pressure. All Toto individuals (30) were homozygous for In/Ins at the DCPI locus. In Bhutia out of 75 individuals about 34% were homozygous for Ins/Ins, 51% were heterozygous (Ins/Del) and 14% were all homozygous for Del/Del. The study revealed that metabolic syndrome can be a major health problem even in traditional ethnic groups.

## PHYSIOLOGY

Study to investigate neuro-chemical regulation of REM sleep and cellular changes after REM sleep deprivation was completed at Jawaharlal Nehru University (JNU), New Delhi. The study investigated a) the involvement of gamma amino butyric acid (GABA) in the laterodorsal tegmentum and pedunculo-pontine tegmentum (PPT) area in the brain (where the REM-ON neurons are located) in REM sleep regulation; b) changes in membrane lipid peroxidation after REM sleep

deprivation; c) whether REM sleep deprivation dephosphorylates Na-K ATPase for increasing its activity. The results reveal that GABA in PPT modulates REM sleep. Synaptosomal membrane lipid peroxidation was decreased after REM sleep deprivation, which was mediated by increased norepinephrine (NE) and the action was mediated through the  $\alpha 1$  adrenoceptors. The results also suggest that calcium plays a role in REM sleep deprivation induced decreased lipid peroxidation. There was a significant decrease in phosphorylated form of synaptosomal Na-K ATPase after REM sleep deprivation, which was again mediated by NE acting through the  $\alpha 1$  adrenoceptor. REM sleep deprivation also increased the transcription of  $\alpha 3$ ,  $\beta 1$  and  $\beta 3$  subunits. The study showed that after REM sleep deprivation there was an increase in the level of phosphatidylethanolamine, however, it was not mediated by NE.

## PHARMACOLOGY

Design and development of new class of antitubercular agents was carried out at Central Drug Research Institute (CDRI), Lucknow with the cell wall of the bacterium as target and based on well known inhibitors. A number of compounds have been synthesized belonging to different prototypes *viz.* glycosylated amino acid, hydroxamates, ureides, alcohols and glycoconjugates; glycosylated cyclopropyl phenyl methanones; glycosylated ureas and C-nucleosides; thiazidine thiones and glycosyl enamines. All the synthesized compounds have been evaluated for antitubercular activity *in vitro* and the selected potent compounds have also been evaluated *in vivo*. Glycosyl amino alcohols and thiazidine thiones have emerged as new class of antituberculars active in MDR strains and *in vivo* also offer new leads for further optimization.

Studies on the antileishmanial properties of novel naphthaquinonoid derivatives and their therapeutic applications were conducted at Jadavpur University, Kolkata to develop diospyrin, a plant-derived lead compound into an effective chemotherapeutic agent. Synthesis, characterization and biological evaluation of novel derivatives (amino-analogues of diospyrin) was achieved. Data indicated variable inhibitory efficacy of the compounds against parasite cells in a dose-dependent manner, the

ethanolamine derivative showing the most marked enhancement in activity as compared to its precursors (*viz.* diospyrin and its diethylether derivative). Evaluation of antileishmanial activity of new derivatives in the infective *L. donovani* strain maintained *in vivo* through serial passages in hamsters showed that the diospyrin derivative achieved nearly 56% inhibition as compared to ~70% inhibition by the standard drug. Combination therapy with diospyrin derivative along with IFN- $\gamma$  on days 14, 16, 18, 20 and 22 post infection in mice showed 44% inhibition, however, 74% inhibition was noted in the infected animals.

## TRADITIONAL MEDICINE

At the Centre for Advanced Research on Drug Development from Selected Natural Products at CDRI, Lucknow clinical trials were undertaken on Picroliv and further studies were conducted on *Centella asiatica* and *Terminalia chebula*.

Studies on *C. asiatica* showed angiogenesis in mice, wound healing activity at different doses in chorio-allantoic membrane model and normal wound healing in guinea pig. It also showed anti-oxidant activity which may have added to the effect. A mixture of *C. asiatica* and curcuminoids also showed wound healing activity in cutaneous punch wound and diabetic wound in rat. Extract and active principles isolated from it showed enhanced learning and memory functions. Fingerprinting and regulatory pharmacological and pharmacokinetic studies were undertaken. Shelf-life and accelerated stability studies of the alcoholic extract and asiaticoside indicated stability for more than 2 yr. Besides studies on the mechanism of action, systemic toxicity and bioavailability studies are ongoing. *Terminalia chebula* showed good anti-stress and anti-oxidant activity.

At the Advanced Centre for Research in Clinical Pharmacology of Traditional Medicine at TN Medical College, Mumbai, and BYL Nair Charitable Hospital, Mumbai studies were undertaken to understand mechanism of action of *Pterocarpus marsupium* as an antidiabetic agent. It has been found to modulate angiogenesis and exert immunomodulatory effect. It protected pancreatic  $\beta$  cells from the damage induced

by streptozotocin (STZ) in rat insulinoma cell lines (RINmf) which appears to be a consequence of anti-oxidant effects. It also restored insulin secretion from STZ damaged pancreatic  $\beta$ -cells although insulin-mimetic actions could not be demonstrated. A novel formulation (ACTM 001) has been found effective in preventing recurrence of diarrhoea in children with malnourishment. ACTM 001 significantly reduced the number of diarrhoeal episodes over a period of 75 days. Further experimental and *in vitro* studies indicated that the three constituent plants prevented bacterial translocation to the mesenteric lymph nodes from the intestine and one of them exerted anti-bacterial effect *in vitro*. A novel herbal formulation from Neem oil (ACTM 002) helped in de-sloughing and hastened wound healing.

A flexible dose multicentric trial was undertaken during 2002-2004 to investigate the blood glucose control in type 2 diabetes with *Vijaysar* (*P. marsupium*) in patients already on an allopathic mono-therapy and to determine adverse effects of this plant. The study was carried out in four centres attached to teaching medical institutions in India representing different segments of the population. Of the 503 patients considered for analysis, 196 completed 20 wk. of treatment. Analysis by the mixed effects model confirmed the anti-diabetic property of *Vijaysar*. Sub-group analysis indicated the need for change of regimen as early by week two of *Vijaysar* treatment for some of the uncontrolled allopathic mono - therapy patients.

Studies on manufacturing, standardisation and quality control of formulations of traditional remedies/natural products at Regional Research Laboratory, Jammu were continued. Raw materials for manufacturing the formulations were collected and standardization and quality control were carried out before dispatching them to Central Biostatistical Monitoring Unit (CBMU) for distribution. Studies were also carried out to reduce the fraction of *P. marsupium* (*Vijaysar*) without changing the profile. The residue on reconstitution continued to have same activity. Chemical markers were identified, four showed hypoglycemic and two hyperglycemic activity. VS - 5 fraction showed additional anti-bacterial and anti-tuberculosis activity. Further studies are being done for validation.

### Survey of Medicinal Plants of Western Ghats

The RMRC, Belgaum has been designated by the Council as the Coordinating unit of multicentric project on survey of medicinal plants of Western Ghats. During the year information for 500 medicinal plants used by local traditional practitioners from the Western Ghats has been collected, along with 497 herbarium sheets of 332 plant species and 514 photographs of 318 plants species. Database development is in progress. The database will provide detailed information on botanical name, synonyms, local names, description of the plant parts used by the traditional practitioners, medicinal uses, formulation, chemical composition, pharmacology/toxicology and clinical trials *etc.*

## HAEMATOLOGY

### HAEMOGLOBINOPATHIES

#### Isolation of Foetal Cells from Maternal Blood for Non-invasive Prenatal Diagnosis of Thalassaemia

Study was undertaken to evaluate the feasibility of development of a non-invasive technology using maternal blood prenatal diagnosis in foetus. So far efforts have not been 100% successful. However in 90% of cases it is now possible to pick up foetal nucleated red cells using a combination of monoclonal antibodies. A well designed nested PCR is able to detect the  $\beta$  thal mutations in the foetal cells.

#### Development and Validation of ELISA for HbA<sub>2</sub>-A Novel Screening Method for $\beta$ -Thalassaemia Carriers

An ELISA has been developed successfully at Genetic Research Centre, Mumbai. The test has great utility in control of  $\beta$ -thalassaemia. It is simple, accurate, inexpensive and precise and suitable for transforming into a user friendly kit.

#### Molecular Characterization of $\alpha\beta$ Thalassaemia and Persistence of Foetal Haemoglobin

Study is ongoing at IIH to understand the basic biology of expression of foetal haemoglobin gene in the adult. Several molecular variants of hereditary persistence of foetal haemoglobin (HPFH) with varying amount of deletion of HbF gene were detected. Studies are ongoing to find out point mutations which lead to HPFH.

## BLEEDING DISORDERS

### Thrombohaemorrhagic Balance in Haemophilia – Implications for Alternative Therapeutic Approach

Coinheritance of thrombophilia genes as a possible ameliorating factor in severe haemophilia was investigated at IHH, Mumbai. Till date 58 haemophilia patients (< 1% deficient factor) have been evaluated, 29 of whom had milder presentation. Around 60% patients with milder presentation had one or more positive thrombophilia markers whereas only 12% clinically severe phenotypes had thrombophilia markers. Studies are ongoing to analyze which of the markers have overwhelming importance in reducing the severity of the disease. In 40% mildly affected patients, thrombophilia markers tested were not exhaustive.

### Thromboelastographic Characterisation of Severe Haemophilia

Global assessment of coagulation system was done by thromboelastography (TEG) of whole blood. Advantage of using the whole blood is that the cellular contribution of coagulation can also be detected. TEG was done in more than 60 haemophilia patients with deficient factor level of < 1%. (Severe by definition). Following patterns were observed: Hypercoagulable (normal), normal with increased fibrinolysis, long latent period followed by split suggesting factor deficiency and no split at all suggesting severe factor deficiency (split was seen in all these patients on addition of antifibrinolytics to the blood). These four patterns have broad clinical correlation. Pattern 1 was clinically mild and has more often co-inherited thrombophilia markers. Pattern 2 and 4 should theoretically improve on antifibrinolytics.

### Genetic Aspects of Essential Hypertension in North East India

Three groups of adult people (>500) from North-East were studied to investigate whether any genetic polymorphism of important genes is linked to hypertension in tea garden workers: i) Tea garden workers with very high prevalence of hypertension (60%), ii) Mizo population with low (<5%) prevalence

and iii) native Assamese population with intermediate prevalence (5-15%) of hypertension. Seven genetic polymorphisms-ACE, AGTR1, CYPIIB2, IC-WT, SF, E-NOS and I-NOS were tested. It was found that ACE D/D gene was strongly linked to hypertension and was also linked in different families. The finding suggested that in many of these patients ACE inhibitors may not be the right choice of first line drugs for hypertension.

### Detection of Factor IX Mutation and DNA Sequencing

More than 100 affected families were tested for mutation of factor IX gene. In 90% Gujarati population with moderately severe haemophilia B, a founder mutation Gly60Ser involving epidermal growth factor domain was found (Fig.12). A mismatch PCR technique has been developed to detect the mutation quickly for quick prenatal diagnosis in these patients. Sequencing

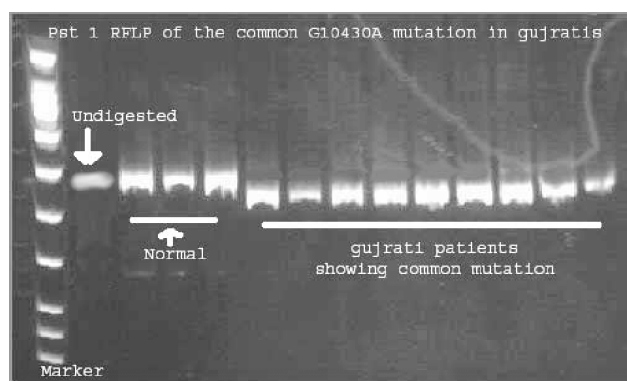


Fig. 12. Mismatch PCR showing Glycoser (GI0430A) mutation in factor IX gene

needs to be done in all these patients to detect the mutation. In 12 cases in whom sequencing could be done, seven different mutations were found.

## HAEMOGLOBINOPATHIES/BLOOD DISORDERS AMONG TRIBALS/OTHER GROUPS

### Jai Vigyan Mission Project on Community Control of Thalassaemia

The Council has recently completed a multicentric project on community control of thalassaemia with

centres in 6 states of the country viz. Punjab, Assam, Gujarat, Maharashtra, Karnataka and West Bengal in which 59,667 cases have been screened. All the centres achieved the target of screening 5000 students. Colleges were selected from different areas of the cities and students from at least 15-30 colleges were screened at different centres. The overall prevalence of beta-thalassemia trait was 2.6% and ranged from 1.6% in Dibrugarh to 3.4% in Ludhiana. The prevalence of Hb E trait was 25.2% in Dibrugarh and 4.1% in Kolkata while occasional cases of Hb E trait were seen in the other 4 centres. A total of 261 students in Dibrugarh were homozygous for Hb E while 74 students in Dibrugarh (1-5%) had Hb E thalassemia. Students with Hb S trait were seen mainly in Vadodara (2.5%) and Mumbai (0.5%) while Hb D trait was more common in Ludhiana (1.2%). In Bangalore, 6 students had beta-thalassemia and 6 sickle cell disease. Few students with thalassemia trait and HPFH trait were encountered at most of the centres with an overall prevalence of 0.1%. There were 33 students who had other structural hemoglobin variants like Hb Q, Hb J and same uncharacterized variants.

There were 73 different caste groups from various regions of the country representing the ethnic diversity in the country. However, in 29 of these castes the total of students were less than 50. Hence the prevalence of different hemoglobinopathies in these groups could be an accurate estimate. Among others, a higher prevalence of thalassemia trait was seen in Vellalas (9.3%), Sindhis (8.6%), Aroras (7.5%) and Lohanas (6.3%). The prevalence of Hb S trait was 15.6% in the scheduled tribes. The prevalence of Hb D trait varied from 2.0 to 4.8% in the Aroras, Prajapatis and Sikhs. The higher prevalence of Hb E trait (94.4% and 38.8%), Hb E disease (8.5% and 11.0%) and Hb E thalassemia (20.7% and 4.8%) were seen in the Chandra Seniya Kayastha Prabhu and Chettiar casters. The other caste groups showing a higher prevalence of Hb E trait were Baidya (9.4%), Billiva (22.2%), Shetty (23.8%) and Chimba (5.2%).

There was a representation of students originating from all the states and Union Territories of India. There were some students from neighboring countries like

Bangladesh (1552), Pakistan (221), Nepal (25), Maldives (10) and Bhutan (4). In most of the states of India, the prevalence of beta-thalassemia trait varied from 1-3%. It was >3% in Andhra Pradesh (3.3%), Haryana (5.4%), Madhya Pradesh (3.8%), Rajasthan (3.1%) and West Bengal (3.9%). The prevalence in students originated from Pakistan was 8.4%. Occasional students with HbS trait were found in many states while the highest prevalence was 3.0% in Gujarat. The prevalence of Hb D trait was >1.0% in Jharkhand (1.7%), Manipur (1.8%), Punjab (1.2%) and the immigrants from Pakistan (2.4%). Hb E trait was most common in the north eastern states of Assam (27.1%), Arunachal Pradesh (18.2%), Meghalaya (10.3%) and Tripura (16.0%). Hb E trait was also seen in students originating from Chandigarh (910.0%), Lakshadweep (14.7%), West Bengal (4.4%) and immigrants from Bangladesh (4.0%). However, Hb E was also seen in several other states in lower frequencies.

The overall prevalence of beta thalassemia trait in antenatal women was found to be 3.0% ranging from 1.3% in Dibrugarh to 4.2% in Kolkata. These women belonged to 72 different caste groups representing genetic diversity. Higher prevalence of beta thalassemia trait was seen in Aroras (9.38%), Sindhis (8.5%), Mandals (7.85%), Pilais (6.25%), Lohanas (6.67%), Jains (4.48%), Kayasthas (4.65%), Khatri (4.6%) and Baidyas (4.35%).

### **Intervention Programme for Nutritional Anaemia and Haemoglobinopathies amongst Primitive Tribal Populations in India**

A multicentric project on intervention for nutritional anaemia and haemoglobinopathies was completed in 16248 individuals from primitive tribes of Maharashtra, Gujarat, Orissa and Tamil Nadu. Sickle cell trait was found in 1.2-22% of the population. Worm infestation and iron deficiency status was also investigated in them and 22-70% of the population was found to be iron deficient. Iron replacement and anthelmintic treatment lead to substantial rise in haemoglobin in these tribal populations. Areas where sickle cell genes are common, nutritional co-morbidities are also prevalent.

### Incidence and Molecular Characterization of G-6-PD Deficiency in North-East India

Up to March 2005, a total of 1696 subjects (748 males and 948 females) in the age group of 4 to 70 yr from Dibrugarh, Sivasagar, Jorhat and Kamrup districts of Assam were screened for G6PD deficiency of which 3% were found G-6-PD deficient. The deficiency in males was 5.5% and in females 1.2%. During April 2005-March 2006, another 996 blood samples belonging to 37 ethnic groups of Assam were screened. The overall deficiency of G-6-PD was found to be 2.1% (male 3.2%, female 1.5%). The ABO blood group pattern among the population was also studied. The most predominant blood group among the study subjects was O (37.3%), followed by B (31.9%), A (22.6%) and AB (8.2%). Negative Rh factor was observed among 1.5% subjects. Further study is in progress.

### Haemoglobinopathies in SC/ST Population in Madhya Pradesh

The RMRC, Jabalpur conducted a study to estimate the prevalence of various haemoglobinopathies in SC and ST populations of Nimar in M.P. Sickle haemoglobin is most common haemoglobinopathy in both tribal and scheduled caste groups ranging from moderate (13%) to high (27%) prevalence (overall prevalence 18%) (Fig. 13). The

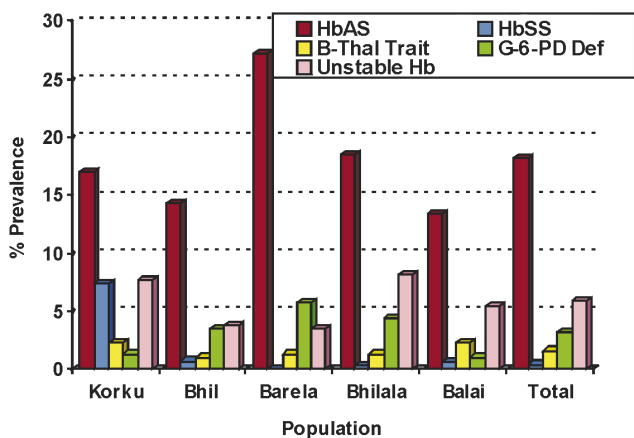


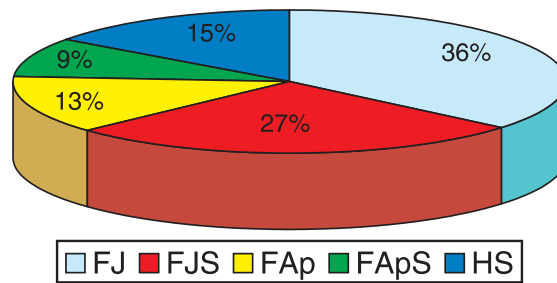
Fig. 13. Prevalence of haemoglobinopathies and G-6-PD deficiency in Nimar

lowest prevalence was observed in Balai SC group (13.4%) and the highest was in Barela tribe (27.2%). The overall prevalence of  $\beta$ -thalassaemia trait in the five population groups under study was 1.6% which varied from 1 to 2.3%.

### Sickle Cell Disease among SC/ST Population of Madhya Pradesh

Sickle cell disease is common in scheduled castes (SC), scheduled tribes (ST) and other backward castes (OBC) of Jabalpur area. Intervention *i.e.* supplementation with folic acid and quick administration of anti-pyretic/anti-inflammatory drugs (as and when needed) along with health education, reduced the severity of the disease considerably. There was reduction in clinical severity of the disease in most of the patients (81%) after intervention.

A study was conducted by RMRC, Jabalpur to know the morbidity profile of sickle cell disease in a total of 350 patients. About 50% of them belonged to SC (Jharia, Mehra, Deharia, etc.) community, 9% to ST and another 20% to OBCs (Kurmi, Lodhi, Yadav, Sahu). Among the main clinical complications for which patients sought medical intervention were painful crisis of bone and joints with fever (85%) and abdominal pain/splenic pain (30%). Splenomegaly was the most common clinical sign reported in 66% of the patients of all age groups (Fig. 14).



**INDEX:** FJ- Fever+ Joint pain, FJS- Fever+Joint pain+Splenomegaly, FAp- Fever+Abdominal Pain, FApS- Fever+Abdominal pain+Splenomegaly, HS-Hepetomegaly+Splenomegaly

Fig. 14. Combination of clinical signs and symptoms in sickle cell disease patients