

Basic Medical Sciences

Basic medical research under the aegis of the ICMR is being carried out at Institute of Pathology (IOP), New Delhi, Institute of Immunohaematology (IIH), Mumbai and through extramural research in various research institutes, medical colleges and universities in the country. Major areas of research include pathology, haematology traditional medicine, pharmacology, biochemistry, immunology, toxicology, physiology, genetics and geriatrics. The Council also handles the Toxicology Review Panel for investigation on new drugs and assists the drug controller General of India in taking decisions on clinical trials/marketing of new drugs in the country.

PATHOLOGY

TUMOUR BIOLOGY

BREAST CANCER

Genetics of Breast Cancer in Indian Population

Studies have been undertaken at IOP, Delhi to elucidate the role of genetic factors in development of breast cancer in Indian population. A Total of 204 breast cancer cases (196 female and 8 male) were screened for mutations in BRCA 1 / 2 genes; 23.5% of them had family history of breast/ovarian cancer and 5.39% had multiple malignancies. Sequence variation in BRCA 1 gene was noticed in 24% patients, 18.36% of whom had family history of breast/ovarian cancer and 73.4% had early onset cancer. Fifty sequence variations were noticed in BRCA2 gene of 19.6% patients, around 5% patients showed variants/polymorphism in multiple exons, 22.5% had early onset. Sequence analysis of DNA fragments showed eight protein truncating mutations including four splice site variants, three recurrent frame shift mutations and one nonsense mutation. Three missense mutations including three polymorphisms novel to north Indian population were also identified.

To study the role of p53 gene in imparting susceptibility to breast cancer and its correlation with other breast cancer susceptibility genes (BRCA1 and BRCA2), screening of genomic and tumour DNA has been initiated.

Study on E- Cadherin Expression in Breast Cancer

E-cadherin (E-CD) is an important cell adhesion molecule shown to be an invasion tumour suppressor gene. Study was undertaken to analyze the expression of E-CD in different morphologic variants of breast cancer as well as to study its prognostic value by comparing its expression with various clinical, histopathological and biological markers. A total of 40 cases of varying histomorphological types were included *viz.* (23 pure infiltrating ductal carcinoma (IDC); 7 pure infiltrating lobular carcinoma (ILC); 7 mixed ductal and lobular type; and 1 each of ductal carcinoma-in-situ (DCIS), infiltrating cribriform and sweat gland carcinoma). The expression of E-Cad antibody was found to be significantly high in cases of pure IDC (60.8%) than in pure ILC (39.2%) cases. Out of 7 mixed cases, 71.4% revealed aberrant expression in both ductal and lobular areas, while in remaining cases positivity was seen in ductal areas only. Invasive cribriform carcinoma case revealed a stronger expression, while negative staining was observed in sweat gland carcinoma. Increased number of high-grade tumour cases (70%) showed aberrant expression. In infiltrating carcinomas, stronger staining was observed in lymph node tumour deposits. Among the other immuno markers employed, a direct correlation of E-cadherin positive staining was observed with ER expression (72.5% cases). Thus, the study revealed that E-cadherin expression has limited value in differentiating high grade IDCs from ILCs, as the expression is lost in all high grade tumours. The expression is stronger in the metastatic deposits as compared to primary tumour.





PROSTATIC NEOPLASIA

Apoptotic and Anti-apoptotic Genes in Neoplastic and Preneoplastic Conditions of Prostate Gland

Study was done to evaluate the role of apoptotic and antiapoptotic genes in neoplastic and preneoplastic conditions of prostate. Total 100 cases of prostatic neoplasia including 55 prostate cancer, 11 atypical adenomatous hyperplasia (AAH) and 34 prostatic intraepithelial neoplasia cases (PIN) were studied for expression of p53, bcl-2, bax and caspase-3 gene and correlation was done with androgen receptor expression. p53 immunoreactivity was found increased as the disease progressed from PIN (26.6%) to cancer (40%). In cases of cancer statistically significant correlation was found with the grade and stage but not with hormone response. In cases of bcl-2 family gene, immunoreactivity for bax gene was found higher in preneoplastic cases (66.6%) as compared to bcl-2 (33%). In cases of cancer bax positivity was 29% (more in localized cancers) while bcl-2 positivity was 18.8%, (more in high grade tumours). A significantly high immunopositivity for bax gene was found in hormone sensitive prostate cancer. Caspase-3 immunoreactivity was found increased in cancer (63.5%) as compared to PIN (44.4%) cases and it also showed significant correlation with hormone sensitive cases. Immunoreactivity for androgen receptors was 60% in cancer prostate and showed no correlation with clinical hormone response.

BLADDER CANCER

Role of In vitro Cytotoxicity Assessment and Immunologic Enhancement in the Management of Superficial Bladder Cancer

Patients having superficial transitional cell carcinoma (TCC) were given intravesical adjuvant chemotherapy and immunotherapy after *in vitro* cytotoxicity assessment on patients' own cultured tumour cells.

A total of 48 cases of superficial TCC were included in the study in whom *in vitro* cytotoxicity assessment had been done followed by intravesical administration of drugs. All these

patients were followed up for evidence of recurrence or metastasis. Among 21 new cases studied majority of cases (60%) responded to single drug. In three cases showing recurrence, repeat cytotoxicity assesment was done.

Study of Host Immune Response in Superficial Bladder Cancer

Study has been undertaken to elucidate the immune mechanism responsible for antineoplastic activity of immunomodulating / chemotoxic agents as well as immunological factors associated with recurrence of superficial TCC of bladder.

Flowcytometric immunophenotyping of PBMC in patients of superficial TCC was done for cell surface markers *viz.* CD3, CD4, CD8, CD25 and CD56 using fluorochrome conjugated monoclonal antibodies. Significant decrease in CD3 and CD4 surface antigens and increase in CD8 and CD25 was noted in cancer patients. No significant difference was noticed in CD56 expression in these patients.

NEURO-ONCOLOGY

Immune Expression of p53 and Vascular Endothelial Growth Factor (VEGF) in Human Gliomas and their Correlation with Proliferation Marker Ki67

At IOP, 50 cases of different histological types of gliomas were studied and graded according to revised WHO classification. Clinical data with reference to age, sex, location were tabulated. Immunoexpression for p53 and VEGF will be correlated with the histological grade and the proliferation marker Ki-67. P53 staining has been completed in all cases while VEGF and Ki-67 are being standardized.

HAEMATOPOIETIC - LYMPHOID MALIGNANCIES

Apoptotic Cell Population as a Marker to Predict Induction of Remission in Patients with Acute Leukemia

Study was carried out to assess the spontaneous ability of acute leukemic cells to

undergo cell death by flow cytometry and to correlate spontaneous apoptosis with clinical response of patients following induction chemotherapy. Thirty one samples were analyzed. Childhood acute lymphoblastic leukaemia (ALL) patients had higher mean apoptotic cell counts while adult ALL patients had higher mean live cell count. Mean live cells were higher than mean apoptotic cells in patients with AML. The mean apoptotic cell count was higher in patients who achieved complete remission (CR) as compared to those who did not achieve CR. Multivariate analysis will be done to see if low apoptosis and high cell viability may be an independent predictor of a poor chemotherapeutic response in patients with acute leukemia.

Expression Pattern of Proliferative and Apoptotic related Proteins in Lymphoproliferative Disorders

Sixty biopsies of lymphoproliferative disorders (35 of non Hodgkin's lymphoma (NHL), 12 of Hodgkin's lymphoma (HD), 11 of reactive lymphadenopathy and 2 of metastatic carcinoma) were included in the study. Of the NHL indolent lymphomas comprised 26%, aggressive 71% and highly aggressive 3%. In NHL, 66% were B-cell and 34 % T- cell type. Primary nodal lymphoma comprised 77% and primary extranodal lymphoma 23%.

The expression of apoptotic associated proteins (Bcl-2 and Bax) and their ratio (Bcl-2 : Bax protein ratio, BBPR) were correlated with immunophenotyping and the histological grade of the tumours. Bcl-2 expression was higher in indolent lymphomas while Bax expression was higher in aggressive lymphomas. The mean BBPR was significantly higher for indolent lymphomas as compared to aggressive lymphomas. Cells with high BBPR tend to survive and those with low BBPR undergo apoptosis readily. The mean proliferative labelling index was significantly lower in indolent as compared to aggressive lymphomas. P53 expression was found to be strongly expressed in 29% of high grade lymphomas. Weak or no expression was seen in low grade lymphomas. Thus, a significant correlation has been found between BBPR and the predicted

biological behaviour of indolent and aggressive lymphomas indicating important role of Bcl-2 and Bax in biological behavior of lymphomas.

PATHOLOGY OF INFECTIOUS DISEASES

CHLAMYDIA TRACHOMATIS INFECTION
Development of Diagnostic Assay for C. trachomatis

A serovar specific monoclonal antibody (Mab) to *C. trachomatis* has been developed which showed greater sensitivity and specificity as compared to existing diagnostic methods. 32.5% symptomatic and 5.6% asymptomatic cervical specimens were found positive by EIA using D serovar specific Mab as compared to DFA (Fig.1). Epitope specificity was done for the developed D serovar specific Mab by EIA using peptides from variable sequences (VS1, VS2, VS3 and VS4) of MOMP for serovar D. The reactivity of core epitope for D serovar specific Mab in VS4 region was high.

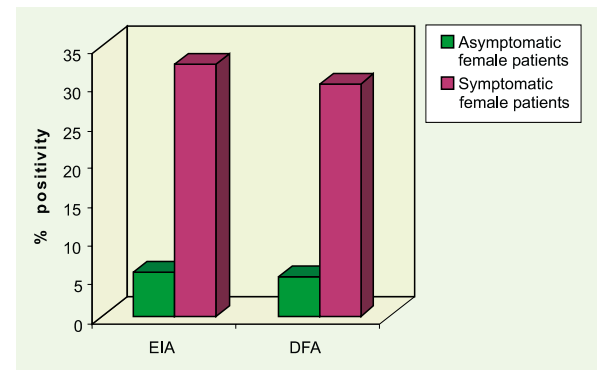
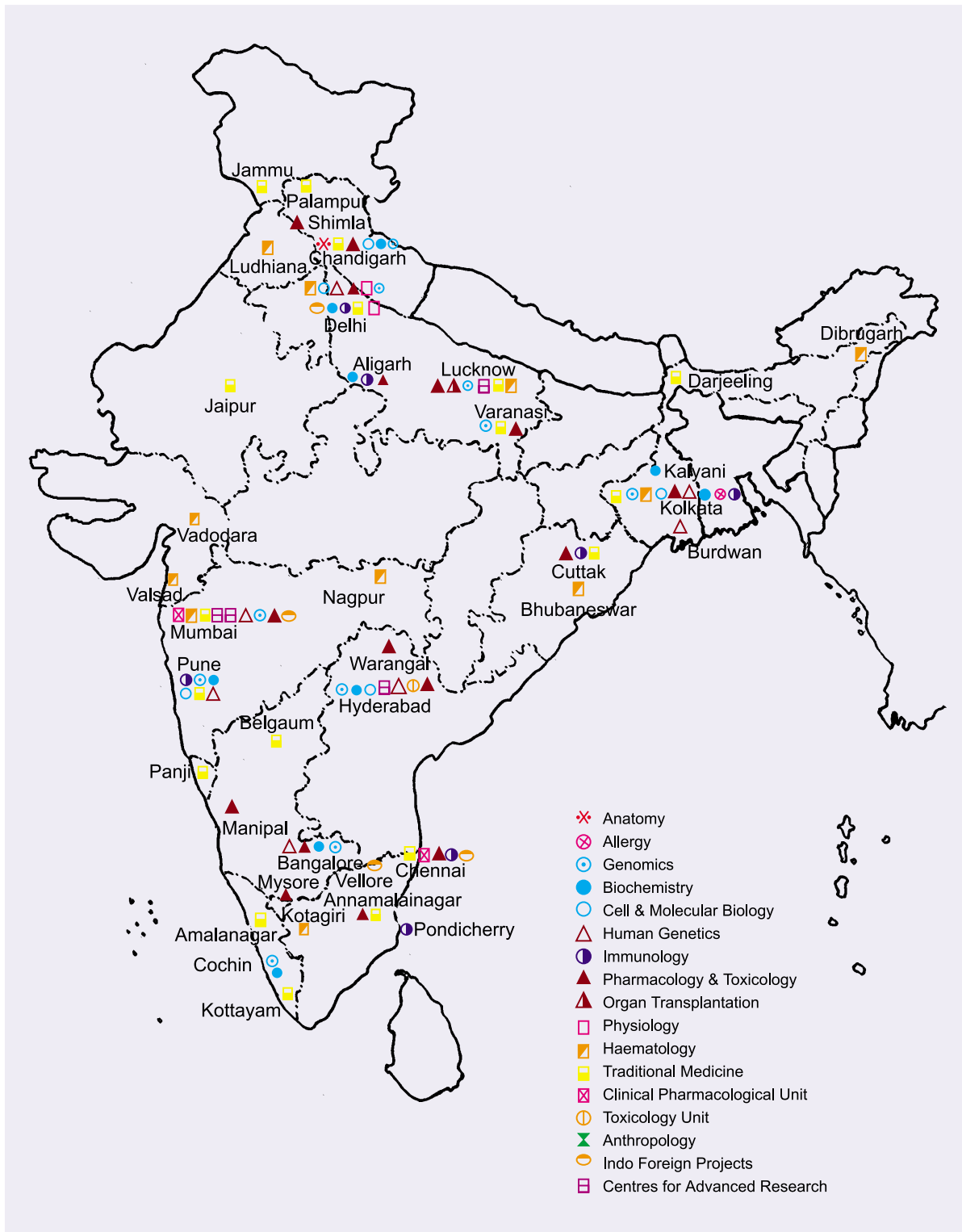


Fig. 1. Prevalence of *C. trachomatis* infection in cervical specimens.

Cytokine Regulation in the Immunopathogenesis of Salpingitis/ Infertility due to C. trachomatis Infection in Women

The production of various Th1/Th2 cytokines in the genital tract (*viz.* cervical secretions and laparoscopic specimens from the fallopian tube) was estimated by ELISA and flow cytometry in *C. trachomatis* positive infertile women after ruling out the presence of few other STD pathogens in the cervix.





Basic Medical Sciences

MAJOR ICMR RESEARCH PROJECTS IN BASIC MEDICAL SCIENCES



By ELISA: Statistically significant decrease in IL-2, IL-6 and IL-12 cytokines was observed in the fallopian tube while the levels of IFN- γ , IL-10 and IL-12 cytokines were significantly upregulated in the cervical

secretions of *C. trachomatis* positive infertile women (Figs. 2, 3).

By Flow Cytometry: IFN- γ , TNF- α , IL-10 and IL-12 cytokines were significantly more often detected in the cervix of *C. trachomatis* positive infertile women (Fig. 4).

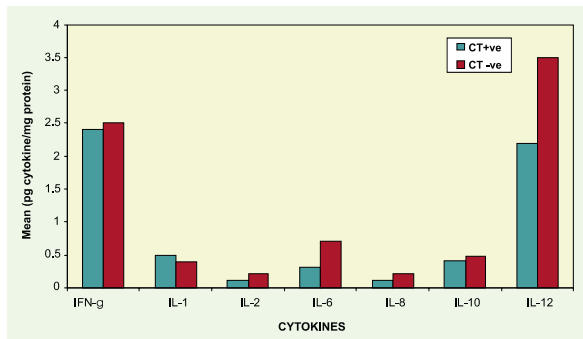


Fig. 2. Cytokine profile in the fallopian tube of *C. trachomatis* positive vs negative infertile women (by ELISA).

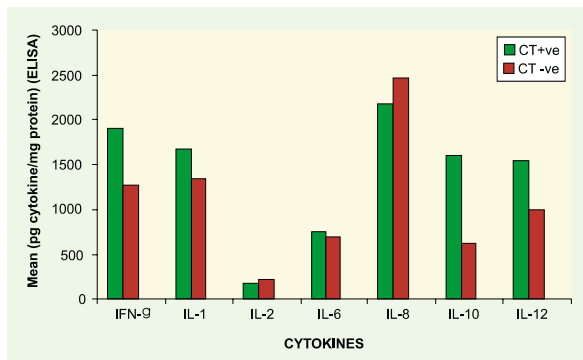


Fig. 3. Cytokine profile in the cervix of *C. trachomatis* positive vs negative infertile women (by ELISA).

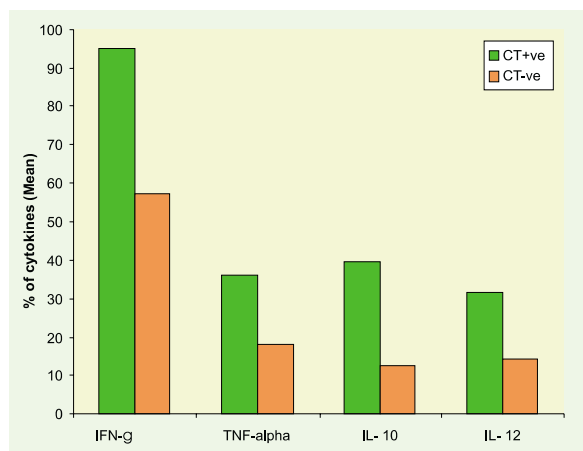


Fig. 4. Pattern of cytokines (IFN- γ , TNF- α , IL-12) in cervix of *C. trachomatis* positive vs negative infertile women (by flow cytometry).

PATHOLOGY OF LEISHMANIASIS

Parasite Detection in Patients with PKDL in India

Evaluation of the sensitivity and specificity of serological, immunohistochemical, and molecular methods in the diagnosis of PKDL was undertaken in 25 confirmed PKDL patients. The diagnostic usefulness of immunohistochemical staining was compared with ELISA with a recombinant (rk39) antigen, and a species specific PCR assay. IHC significantly enhanced the sensitivity of detection (88%) of PKDL over routine H & E staining. ELISA with a recombinant antigen provided an economical and practical assay with 96% sensitivity. PCR was found to be the most sensitive and specific diagnostic method. The tests would facilitate diagnosis of patients with PKDL enabling their timely treatment.

Nested PCR Assay for Detection of *L. donovani* in Slit Aspirates from PKDL Lesions

A nested PCR assay to detect parasite DNA in slit aspirates from skin lesions of patients with PKDL was developed. PCR results were positive in 93% samples by nested PCR assay, while only 69% were positive in a primary PCR assay. The nested PCR assay allows reliable diagnosis of PKDL in a noninvasive manner.

Centrin Knockout Mutants

Centrin is a calcium binding cytoskeletal protein involved in duplication of centrosomes in higher eukaryotes. In order to explore its role in this protozoan parasite, *Leishmania* deficient in the centrin gene (*LdCEN*) was created at IOP, Delhi. These centrin null mutants (*LdCEN*^{-/-}) showed selective growth arrest as axenic



amastigotes but not as promastigotes. Flow cytometry analysis confirmed that the mutant axenic amastigotes have a cell cycle arrest at the G2/M stage. The axenic amastigotes also showed failure of basal body duplication and failure of cytokinesis resulting in multinucleated 'large' cells. Growth of *LdCEN*^{-/-} amastigotes in infected macrophages *in vitro* was inhibited and also resulted in large multinucleated parasites. This is the first report where disruption of a centrin gene displays stage specific/cell type specific failure in cell division in a eukaryote.

Genomic Microarray based Identification of Differentially Expressed Parasite Genes in PKDL

Studies were undertaken to identify genes differentially expressed in *Leishmania* parasites isolated from PKDL patients in comparison with those from kala-azar patients, using the highly sensitive microarray technology. These gene products are most likely candidates responsible for the different clinical manifestations in case of PKDL. Overexpression of gp63, gp46 and calpain like protease was established in PKDL. Such differentially expressed genes may hold the key to understanding of the parasite genetic factors that regulate the persistence after clinical cure of VL.

OTHER STUDIES

Monitoring of Organic Chemical Pollutants in Placental Tissue

Study was conducted at IOP, Delhi with the aim to establish the utility of human placenta as a tool for comprehensive bio-monitoring for organic pollutants. Samples collected from a total of 45 women (30 random and 15 exposed to agricultural chemicals during pregnancy) were analyzed.

The results revealed presence of pyrethroid, an active ingredient of commonly used mosquito repellent in significantly large number of samples. Some other organo-chlorine and organo-phosphorus compounds were also detected. Further extraction, analysis and reconfirmation of observed compounds is being carried out.

In vitro Cultivation of Differentiated Human Epidermis for Autologous Grafting in Burns

The method to culture multi-layered differentiated epidermis was standardized using Rheinwald and Green culture conditions. The cultured epidermis was applied on limited areas (less than 500 cm²) in two patients. In one patient the take was about 70% of the grafted 65 cm² area and in the other it was 100% of the total grafted 400 cm² area. Grafting in larger than 500 cm² area is being attempted.

Flowcytometric Evaluation of Estrogen Receptors in the Ejaculated Human Spermatozoa and Germ Cells of Rats

Earlier studies undertaken at IOP demonstrated the immunohistochemical localization of estrogen receptors (ER) and a number of estrogen modulated proteins in the ejaculated human spermatozoa.

During the year studies were undertaken to investigate the localization of ER in spermatozoa using flow cytometry. Preliminary studies have indicated that the ER can be evaluated flowcytometrically. (Fig. 5).

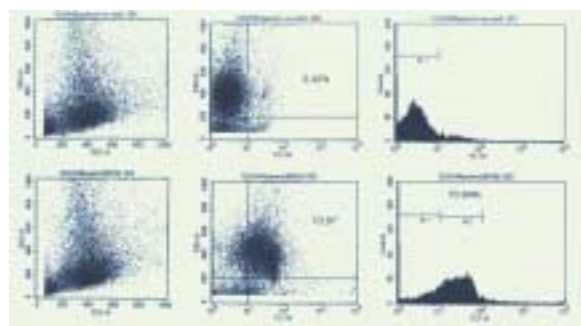


Fig. 5. Flow cytometric separation of ER α labeled human ejaculated spermatozoa. The top panels show the control and the lower two ER α labeled with FITC. The % of unlabeled/labeled sperms are represented as M1 and M2.

Studies have also been undertaken to evaluate the presence of splice variant of ER and PR, if any, in human spermatozoa from fertile subjects and to investigate correlation of the same with male infertility.

Preliminary results show localization of ER α in different germ cell populations of rat testes (Fig.6)



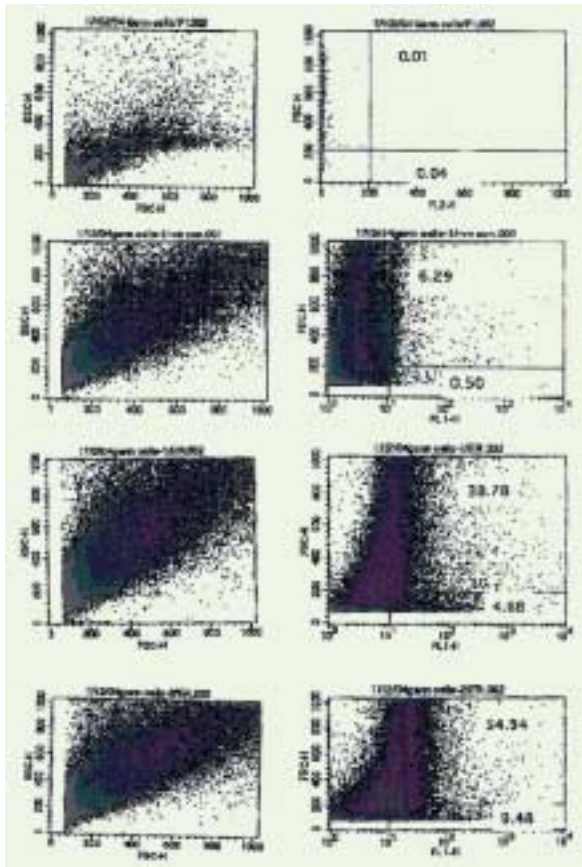


Fig. 6. Flow cytometric separation of ER α labeled rat testicular germ cells. The top two panels indicate the controls and the lower two FITC labeled ER α samples.

BIOCHEMISTRY

A study on the transcriptional regulation of human LDL-receptor gene by cholesterol has been concluded at PGIMER, Chandigarh. The study revealed that transmembrane signalling through cholesterol specific 69 kDa receptor (Ck) was involved in transcription regulation of 160 kDa apoprotein B specific LDL receptor gene.

In another project, effect of prenatal exposure of ethanol on the functional development of intestine in rats was studied at Punjab University, Chandigarh. The litter size was reduced, gestation period delayed and pups born to ethanol fed mothers exhibited low body and intestinal weight and intestinal length during postnatal period. Aldolase dehydrogenase activity after 3-4 weeks of birth and transport of glucose, glycine and leucine in intestine was reduced in ethanol exposed pups. Absorption and

binding of IgG and activities of brush border sucrase, lactase and leucine aminopeptidase were also reduced in intestine of pups born to rat mothers fed ethanol during gestation. The findings suggest that prenatal ethanol exposure modifies intestinal development after birth in rats.

Study of protective efficacy of *M. tuberculosis* complex specific protein antigens against experimental tuberculosis was carried out at PGIMER, Chandigarh. Preliminary results suggest that supplementation of BCG with immunodominant antigens (*i.e.* *M. tuberculosis* complex specific proteins) enhances the immunogenicity of BCG and hence may potentially increase its protective efficacy.

Other ongoing studies include development of antigen detection based diagnostic technique for tuberculosis, understanding molecular basis of pathogenesis of tuberculosis and development of peptide based immunogens from different stages of *P. vivax*.

IMMUNOLOGY

Immunological and molecular studies on the mosquito humoral factors involved in the susceptibility/regulation of *W. bancrofti* infection and development in *C. quinquefasciatus* is ongoing at VCRC, Pondicherry. Analysis of haemolymph samples from infected, normal and naïve mosquitoes indicated six proteins (two new proteins of 40 and 22 kDa, three proteins of 7, 14 and 70 kDa and one protein of 110 kDa).

Study to understand the role of sialoglycoconjugates in host-parasite interactions in visceral leishmaniasis is ongoing at IICB, Kolkata. During the year 13 clinical isolates procured from the Kala-azar Research Centre, Varanasi have been preserved in Leishmania Bank and their *in vitro* anti-leishmanial drug responsive profile has been studied. Identification of different derivatives of sialoglycans on *L. donovani* has also been done.

PHYSIOLOGY

A simultaneous electroencephalography (EEG) and MRI study of sleep-wakefulness in



rat brain has been concluded at AIIMS, New Delhi. Under the study a stereotaxic restraining assembly was designed and developed for simultaneous electrophysiological recordings and functional MRI (fMRI) data acquisition from a conscious rat. Non-magnetic stereotaxic apparatus thus designed facilitated the restraining of head and body of the unanesthetized conscious animal during MRI experiments.

The apparatus was used for carrying out simultaneous electrophysiological and fMRI acquisitions during sleep-wakefulness in a 24-hr sleep deprived rat. Functional data shows activation of preoptic area (POA) and deactivation of fronto parietal cortex during sleep as compared to wake state. Such experiments are useful in characterizing the functional state of different brain regions with good temporal and spatial resolution and are useful in studying different neurophysiological mechanisms like sleep and seizure activity. This study demonstrates the feasibility of carrying out simultaneous electrophysiology and fMRI experiments from conscious animals.

Another study on physiological and behavioural aspects of human circadian rhythm under prolonged socio-temporal isolation was accomplished at Madurai Kamaraj University, Madurai. Sleep, wake and rectal temperature rhythms of eight subjects (age 20-22 yr) under societal conditions showed 24h periodicity.

In two separate experiments a newly married couple (32 and 23 yr old) and a mother (41 yr), son (14 yr) and daughter (12 yr) lived together inside the Human Isolation Chamber (HIC), where all facilities except information on time were available. The couple stayed in the HIC for 29 days and showed circadian periodicity of more than 24h in their sleep - wake and temperature rhythms including a week during which they had their wristwatches indicating that mere knowledge of time is an inadequate zeitgeber. Similarly family members (stayed 14 days) also showed circadian patterns in their sleep, wake and temperature rhythms with more than 24h periods. All the five subjects showed close to 24h periods under societal conditions, recorded both during pre-and post isolation.

Their 2h estimations positively correlated with the duration of wakefulness during isolation indicating that short and long time estimates are based on different mechanisms. The timings of sleep and wakefulness for three infant (two boys and one girl) subjects were noted unobtrusively by their parents, from birth to 5-6 months of age. At early ages there were several bouts of sleep and wake components and the infants exhibited circadian rhythm in their sleep-wakefulness. After 14-15 weeks of age the rhythm became apparent circadian. The numbers of bouts of sleep and wakefulness decreased with increasing ages. However, the duration of sleep was still greater than that of wakefulness at the age of six months. Thus a clear circadian pattern of sleep and wakefulness was not yet evident at the age of six months.

GENETICS

Genetic causes of Male Infertility

In a study for establishing genetic causes of male infertility in India at Centre for Cellular and Molecular Biology (CCMB), Hyderabad a total of 209 infertile males (age 26-42 yr) were included. Analysis of cytogenetic and hormone profile, Y-chromosome, Southern hybridization, POLG gene, DAZ gene cluster and DAZL gene was undertaken. Y chromosome microdeletion and rearrangement was seen in 11.5% men. Deletion of AZF regions and AZFb and AZEc regions was detected in 12 azoospermic samples and in 4 of them the deletions were very large involving also the heterochromatin region. CAG repeats in the mtDNA polymerase gene (POLG) were found to be involved in male infertility. Around 83% azoospermic men had homozygous wild type allele. Deletion of one or more copies of DAZ gene was seen in 63 men, 14.52% of whom were azoospermic, 25% were oligospermic and 12.5% were oligoteratozoospermic. Novel mutations in DAZL gene were observed in 5 azoospermic men.

Single Nucleotide Polymorphisms (SNP) in ICAM1 & TNF α among the Jarawa in A & N Islands

A survey of DNA sequence variation in two functionally coding regions *i.e.* genes coding



for tumor necrosis factor α (TNF α) and intercellular adhesion molecule 1 (ICAM1) was conducted among *Jarawas*, a negrito tribe of Andaman & Nicobar Islands by the RMRC, Port Blair. Both these genes play important roles in a variety of human diseases. Genomic DNA was isolated from blood samples of 35 *Jarawas* and amplified and sequenced. A total of six polymorphic sites were detected in the TNF- α gene, of which two single nucleotide and one insertion/deletion (indel) polymorphisms present in gene have already been reported. Three new polymorphisms have been detected in *Jarawas*.

The only exonic (exon4 and 3' UTR) SNP found in TNF- α harbours an A to C transversion while the remaining five changes lie either in upstream regions of the gene or in intron.

Four SNPs were detected in genomic region coding ICAM1. Like the SNPs present in TNF- α , the frequencies of ICAM1 SNPs are also moderate to high. A total of eight haplotypes were observed, of which HT-1 and 2 are equally frequent. Except for one haplotype (HT-8), all haplotypes occur more than once in *Jarawa* gene pool. All ICAM1 haplotypes can be joined sequentially by single site mutational difference, though the possibility of recombination in this gene cannot be ruled out. The major observations emerging from the pattern of sequence variability of TNF- α and ICAM1 genes in *Jarawas* are: (1) there is low DNA sequence variability, (2) rare SNPs are absent, (3) the group harbours new SNPs, and (4) there is restricted variation in haplotypes.

Mobile Workshops in Genetics

Mobile workshops were organized in Maharashtra for students in human genetics.

HANDIGODU SYNDROME

Handigodu syndrome is a peculiar disease of the osteoarticular system found in Shimoga and Chikmagalur districts of Karnataka amongst the *Chennagi* and *Chaluvadi* Scheduled Caste communities. Earlier studies could not attribute the disease to toxicity or nutritional deficiency

and stressed need for identification of the gene responsible for the disease. The localisation and characterisation of the gene may provide insight into its pathophysiology and role of calcium nutrition on the course and progression of the disease. Study is being carried out at Bangalore and Mumbai for identifying families with Handigodu disease for mapping and localizing putative gene and for evaluating suitable intervention programme for management of the disease.

The pedigree charts of all the families have been studied and in many of them all 3 generations were found to be affected. Age of onset of disease symptoms was found to be 20-60 yr and atleast one affected parent was identified. The prevalence amongst males and females was found to be equal and almost 50% family members were affected confirming the autosomal dominant pattern of inheritance. The radiological changes were predominantly present in hip joints and spine. The primary change is dysplasia of femoral head epiphyses, usually bilateral. Spinal changes also occur along with changes in knees and wrist joints.

ANTHROPOLOGY

Study on determinants of genetic and socio-cultural aspects of health (*vis-a-vis* role of health providers) among the tribes of coastal, desert and Himalayan regions was carried out at University of Delhi, Delhi. *Dhodias* of Valsad, *Bhils* of Barmer and *Kinnauras* of Kinnaur were found to be in different stages of demographic transition gradually progressing for a better future. *Dhodias* can be considered as most progressive followed by *Kinnauras* and *Bhils*. Morbidity trends were not very clearly defined amongst them. Malaria was not recorded among *Kinnauras* at all but was rampant among *Dhodias* and was seasonal among the *Bhils*. The HbS had a high frequency among *Dhodias* but was not found in *Bhils* and *Kinnauras*. *Dhodias* and *Bhils* also revealed high frequency of problems related with oral cavities, while *Kinnauras* showed comparatively high frequency of fracture and problems related with vertebral column



because of heavy load lifting on back due to steep terrain. Few cases of fluorosis were recorded among *Bhils*. Tuberculosis was more prevalent among *Bhils* and *Dhodias*. Other morbid conditions (water borne and communicable diseases, skin diseases, infections *etc.*) were common among all with varying frequencies.

All the three tribal populations had very strong preference for utilizing the services of traditional healers. These were "*Bhagat-Bhua*" in *Dhodias*, "*Bhope*" in *Bhils* and "*Amchi/Lama*" in *Kinnauras*. As far as providers were concerned, the best situation was observed among *Dhodias* — large number of private practitioners with recognized medical degree were practicing among them. No private practitioners were there in *Kinnauras* and very few in *Bhils*. Government health providers were doing stupendous job in all the three districts. However, scattered populations in Kinnaur and Barmer due to hilly terrains and desert conditions, poor transportation/connectivity and vacant position at various levels hampers effective health care delivery. *Dhodias* also had the highest female literacy but were economically not affluent whereas *Kinnauras* are economically affluent as well as literate. *Bhils* in comparison are neither literate nor economically affluent. Road transport leading to connectivity with the providers is most efficient in *Dhodias* but the *Kinnauras* and *Bhils* have poor connectivity. It can be concluded that health providers are the most important factors in improving the health status of the tribal groups in India.

GERIATRICS

Advanced Centre for Studies on Biochemical Correlates of Aging

Studies were undertaken at University of Hyderabad, Hyderabad to understand the molecular mechanisms of aging process in brain, establish molecular markers for aging process and to take up interventions to retard the progress of aging and certain age associated diseases.

TRADITIONAL MEDICINE

The Centre on Pharmacological Research for Drug Development from Plant Sources in Selected Traditional Remedies at CDRI, Lucknow is focussing on hepato-protective effect of *Picroliv*, wound healing activity of *Centella asiatica* and adaptogenic activity of *Terminalia chebula* as well as its chemical fingerprinting.

Clinical trials and pharmacokinetic studies with Picroliv revealed that response to picroliv therapy is good with no side effects. Phase III double blind multicentric clinical trials in patients with alcoholic cirrhosis and tuberculosis receiving MDT have been initiated at KEM Hospital, Seth GS Medical College, Mumbai. Chemical fingerprinting of *Picroliv* has identified seven compounds *viz.* Catalpol, Piccin, Androcin, Minecoside, 6-O-Feruloyl Catolpol, Cucurbitacin Glucoside-13 and Cucurbitacin Glucoside-21.

Studies on the effect of *T. chebula*, *Panax ginseng* and its active compound K022 on acute and chronic stress revealed that all these compounds possess potent adaptogenic activity but K022 is more effective than standard *P. ginseng*.

Studies on effect of *C. asiatica* fresh extract on learning and memory and anti-stress activity revealed its potential as memory enhancer. Chemical fingerprinting and stability studies with marker compounds asiaticoside -C and madicassodide revealed that the former was stable for more than two years.

At the Centre for Clinical Pharmacology of Traditional Medicine at BYL Nair Hospital, Mumbai studies are continuing on *Pterocarpus marsupium*, medicated oral rehydration solution (ORS) and Neem oil.

Study is being carried out to elucidate the mechanism of action of *P. marsupium* using experimental aspects such as protection against beta cell damage, effect on angiogenesis, immunomodulatory effects and insulin mimetic activity.

Evaluation of medicated ORS, an *Ayurvedic* formulation containing *Cyperus rotundus* (*Nagarmotha*-whole red kidney beans), *Zinziber officinale* (*Sunthi*) and *Cuminum cyminum* (*Jeerak*) (Fig. 7), was done experimentally and





Cyperus rotundus - root



Zingiber officinalis – rhizome



Cuminum cyminum - seed

Fig. 7. Constituents of medicated oral rehydration solution.

clinically in diarrhoea. The activity of medicated ORS was studied *in vitro* and *in vivo*. Effect of crude extract of *C. rotundus* reduced bacterial translocation in intestine. Only hot extract of *Zingiber officinale* showed dose dependent antibacterial activity. It is planned to isolate and identify the most common organisms from stool samples and test the effect of plant extracts on them.

Clinical and *in vitro* studies were conducted with in-house prepared *Neem* formulation, *Neem-b-heal* (commercial) and glycerine-acriflavin (conventional) preparation for its wound healing effect.

Composite wound score and wound area decreased more with in-house *Neem* oil

preparation. The best effect of *Neem* was seen on the granulation tissue formation, rate of formation and collagen deposition (Fig. 8). A herbal formulation containing *Neem*, *Aloe vera* and *Calendula officinalis* showed significant increase in angiogenesis.

Studies carried out at AIIMS, New Delhi in experimental models of Alzheimer's disease in rats suggested that aqueous extracts of *C. asiatica* and *Celastrus paniculatus* can be used as cognitive enhancers in diseases associated with dementia and oxidative stress like Alzheimer's, Parkinson's, Huntington's, Pick's diseases *etc.* *C. asiatica* and *C. paniculatus* significantly prevented the cognitive impairment, cholinergic deficit and oxidative stress induced by brain glucose



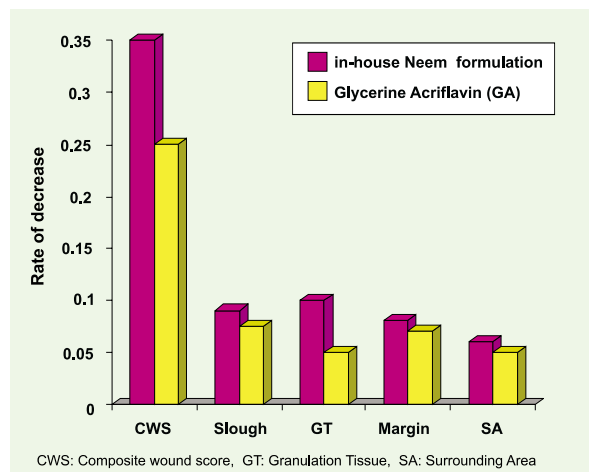


Fig. 8. Rate of decrease in wound score with neem oil formulation.

metabolism impairment in ICV streptozotocin treated rats. The results are in accordance with previous reports that *C. paniculatus* enhances learning and memory.

At the ICMR unit on standardisation, quality control and formulation of traditional medicines at National Institute of Pharmaceutical Education and Research (NIPER), Mohali studies were done on *Streblus asper*, *Crataeva nurvala* and *P. marsupium*. Isolation and purification of alkaloid (cadabacine) from *C. nurvala*, strebloside, mansonin, cannodimethoside and asperoside from *S. asper* and pterostilbene, liquiritigenin, marsupin and propterol from *P. marsupium* (Fig. 9) has been done so far.



Fig. 9. *Pterocarpus marsupium* tree.

CLINICAL PHARMACOLOGY

The Clinical Pharmacology Unit at TRC Chennai carried out studies on malabsorption of rifampicin and isoniazid in HIV infected patients with and without tuberculosis; bio-availability of rifampicin and other anti-tuberculosis drugs in patients with advanced HIV; analysis of generic antiretroviral formulations manufactured in India; vitamin A levels in sputum positive pulmonary tuberculosis patients in comparison with household contacts and healthy 'normals'; standardization of the method for the estimation of ethambutol in pharmaceutical preparations and biological fluids and antibacterial and antimycobacterial activities of compounds from fruits of *Piper longum*.

The study on rifampicin and isoniazid absorption in HIV infected patients with and without tuberculosis revealed malabsorption of these drugs in them. Significant correlation between CD4 counts and urinary level of rifampicin was seen. The results of the study further suggested that D-xylose absorption test in urine can be used to screen HIV infected patients who may not be absorbing rifampicin and isoniazid adequately.

Analysis of generic antiretroviral formulations manufactured in India by Aurobindo Pharma, Ranbaxy and Cipla indicated that the amount of active drug is very similar to medications manufactured in the USA.

Study of vitamin A levels in sputum positive pulmonary tuberculosis patients revealed that vitamin A deficiency is common among them. The study further showed that levels returned to normal following anti-TB treatment (Fig. 10) suggesting that vitamin A supplementation may not be necessary. An association was seen between serum vitamin A levels and sputum smear gradation in patients at the start of treatment.

A simple and accurate method has been developed for determination of ethambutol in urine and in pharmaceutical preparations containing ethambutol alone and in combination. Sensitivity, reproducibility, recovery and stability of the method were found to be very good.



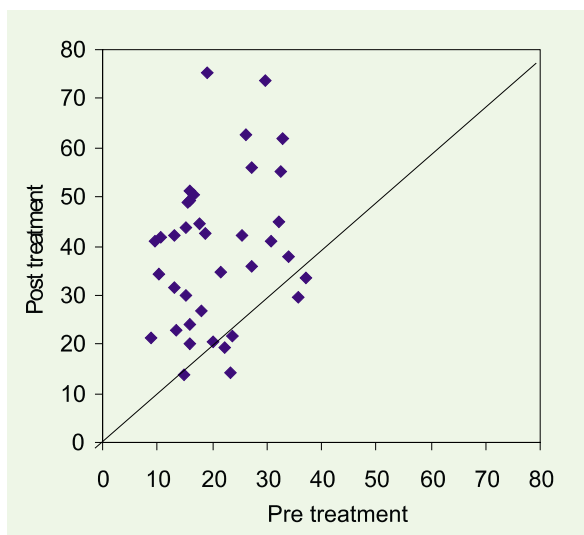


Fig. 10. Serum vitamin A levels at start and end of treatment in pulmonary TB patients.

Studies were continued at Clinical Pharmacology Unit at Seth G.S. Medical College and KEM Hospital, Mumbai for assessing the safety and efficacy of primaquine vs bulaquine as gametocidal agents in *P. faciparum* malaria in adults. It was found that at higher doses (60 to 240 mg) bulaquine was more effective than primaquine.

Studies on therapeutic drug monitoring of lamotrigine in epileptic patients was completed to evaluate the relationship between plasma concentration of lamotrigine and its efficacy and toxicity and to identify the therapeutic range of lamotrigine in Indian population.

Studies on menstrual cycle and hormonal changes in women receiving antiepileptic therapy and evaluation of effect of anti- epileptic drugs on bone mineral density and biochemical markers in young Indian epileptic patients are ongoing.

PHARMACOLOGY

Evaluation of the effect of exposure of rats to a pyrethroid based mosquito repellent (containing allethrin) during development was carried out at Industrial Toxicology Research Centre, Lucknow. Neurochemical and neurobehavioural studies indicated that inhalation during early life may lead to significant abnormalities in infants by affecting the CNS. Damage to the brain was long lasting.

Further oxidative stress levels in different regions of the brain in pups after prenatal, postnatal and perinatal exposure resulted in significant alterations.

Drug Development through Genomics

In a project completed at CDRI, Lucknow cloning of DNA ligase gene from *Mycobacterium* using PCR technique was done using host strain of *E. coli*.

PRECLINICAL TOXICOLOGY

The Pre-clinical Toxicology Unit at NIN, Hyderabad continued pre-clinical safety assessment of pharmaceuticals having potential therapeutic efficacy; safety assessment of pharmaceuticals having toxic actions on specific organs; pharmacokinetic/pharmacodynamic profile of drugs and regulatory pharmacology.

ORGAN TRANSPLANTATION

Fetal neural cells remain the most widely used graft source for transplantation in rat model of Parkinson's disease. However, low survival and less availability of the fetal tissue as well as the ethical issues associated with use of fetal grafts hinder this approach. These limitations led to development of newer techniques using paraneural cells as substitutes for dopamine and to provide trophic support. Study on cellular and functional recovery of Parkinsonian rats after intrastriatal transplantation of paraneural cells [carotid body (CB) and olfactory ensheathing cells (OEC)] was conducted at ITRC, Lucknow.

Paraneural cell aggregates were co-transplanted with fetal ventral mesencephalic cells (VMC). Significant functional restoration in co-transplanted group was seen compared to individual VMC or OEC transplanted group. The results suggest that OECs are rich in various neurotrophic factors while CB cells release large amount of dopamine in hypoxic conditions due to which they can be used as alternate to fetal VMC. Moreover, these cells are also rich in various neurotrophic factors thereby helping in increased survival of transplanted VMCs as well as host dopaminergic neurons.



HAEMATOLOGY

BLEEDING DISORDERS

Risk Factors for Coronary Artery Disease

The project ongoing at IIH, Mumbai brought out a very important association of hyperhomocysteinaemia with coronary artery disease leading to myocardial infarction. Of the total patients studied, 55% were found to have hyperhomocysteinaemia. In 60% of them folic acid therapy lowered the homocysteine levels significantly. It remains to be seen whether folic acid therapy on long term basis can prevent myocardial infarction in these patients.

von Willebrand Disease (VWD)

VWD is an autosomally inherited bleeding disorder. Studies undertaken at IIH revealed that in ~12% of the patients idiopathic menorrhagia occurs due to VWD and in 8% it occurs due to an ancillary coagulation disorder. Antenatal diagnosis of severe VWD by using DNA based technology has been established at this institute.(Fig.11)



Fig. 11. Prenatal diagnosis in type 3 VWD family.

Hemophilia

Establishment of Prenatal Diagnosis in Hemophilia A and B

Till date prenatal diagnosis has been offered at IIH, Mumbai in 300 pregnancies to patients coming either in first or second trimester from all over India. Affected families were counselled and all of them opted for medical termination of pregnancy. It is an important achievement of IIH as in developing countries it is one of the

main approaches of controlling this serious heritable bleeding disorder.

PCR Technique for Detection of Factor IX Gene Mutations

A multiplex PCR technique has been established at IIH to detect mutation for factor IX deficiency. Of the tested patients 10.5% showed the presence of a deletion. This technique will be useful for prenatal diagnosis in families of sporadic cases of hemophilia B.

Human Leucocyte Antigen (HLA) in Chronic Hemophilic Synovitis

Studies were undertaken in 400 hemophilia patients to see whether chronic synovitis in these patients was linked to any particular HLA antigen. Sixty four percent hemophilia patient with chronic synovitis were found to possess HLA – B27 antigen indicating that the risk of getting chronic synovitis in patients having HLA-B-27 is >12.

Molecular Basis of G6PD Deficiency in India

In studies undertaken at IIH, it was found that besides G6PD Mediterranean there are other variants like G6PD Orissa and G6PD Kerala - Kalyan in our country responsible for drug induced hemolysis and neonatal jaundice.

Platelet Antigen Polymorphism in Newborns in India

Molecular technique to detect human platelet alloantigen system (HPA 1 – HPA 8) was standardized at IIH. The population distribution of these antigens in Western India has been worked out. Several neonatal alloimmune thrombocytopenic purpura (NAITP) patients were detected and helped by proper intervention.

HAEMOGLOBINOPATHIES

Jai Vigyan Mission Mode Project on Community Control of Thalassemia

The multicentric project on community control of thalassemia syndromes aims at education, screening, counselling and identifying



couples at risk of having thalassemic children so as to prevent birth of these children and to develop a national referral centre for control of thalassemia major in the country. Study is being carried out on college students and pregnant women in Maharashtra, Gujarat, Assam, West Bengal, Punjab and Karnataka.

A total of 21645 cases (10673 college students and 10972 antenatal cases) have been screened till date. The results showed that majority of students had no knowledge about the disease. In Kolkata and Vadodara centres 40-50% students knew only the name of the disease. The maximum percentage of iron deficiency was seen in females from Dibrugarh (40%) and Kolkata (27%). The prevalence of β -thalassemia trait varied from 2 to 4%, Hb E was predominantly seen in Dibrugarh and Kolkata whereas HbD was found in Ludhiana. The prevalence of anemia varied from 78% to 93%. Iron deficiency based on FEP levels ranged from 29 to 41%. The prevalence of iron deficiency varied from 17-69% in β -thalassemia carriers.

Molecular Characterization of $\delta\beta$ Thalassemia and Hereditary Persistence of Foetal Haemoglobin (HPFH)

A gap PCR approach was used for molecular characterization of patients who were phenotypically characterized for thalassemia. Eight out of 30 cases were heterozygous for 48.5 Kb Indian deletion (HPFH-3), 11 were heterozygous for Asian Indian inversion deletion $G\gamma$ ($Ar\delta\beta$) thalassemia and 4 were found to be heterozygous for Vietnamese deletion (12.5Kb). Seven cases still remain uncharacterized.

POPULATION GENETICS

Molecular Characterization of Bombay Phenotype

Thirty eight serologically confirmed Bombay phenotype samples were investigated for detecting the T 725 G mutation in the H (FUT 1) gene. All samples except two showed T 725 G mutation.

STEM CELL BIOLOGY

Umbilical Cord Blood Stem Cells and their Expansion *ex vivo*

Different conditioning media (PCM, GF 1, GF 2 and stromal cells +GF2) were used for *ex vivo* expansion of CD 34 +ve umbilical cord cells at IIC. It was found that GF 2+ stromal cells provided the maximum expansion (10 fold) over 14 days (Fig. 12). Besides this cryopreservation of stem cells with different agents was tried and an optimum cryoprotectant mixture for this purpose has developed.

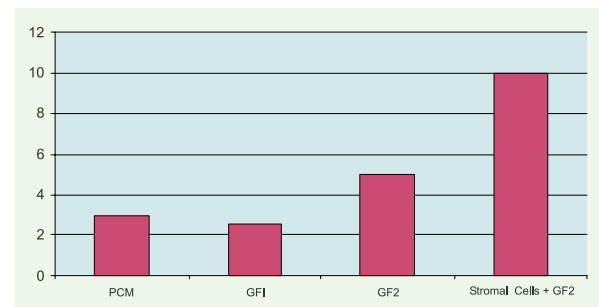


Fig. 12. Total expansion of CD34 in umbilical cord stem cells with different media.

OTHER STUDIES

Cytogenetic Factors in Myelodysplastic Syndrome (MDS)

In India most of the patients of MDS present as young adults (20-40 yr). Cytogenetic studies and mitomycin C test (for chromosomal fragility) was conducted on 27 MDS patients. Atypical cytogenetic abnormalities were seen in 40% (Fig.13 & 14) and increased chromosomal breakages in 45% of these patients.

HLA Association with HIV-1 Infection

HLA class I allele frequency was tested in 38 HIV-1 positive patients and compared with 120 matched controls. HLA B*3520, B*1801 and Cw*1507 alleles were found to be uniquely associated with HIV.



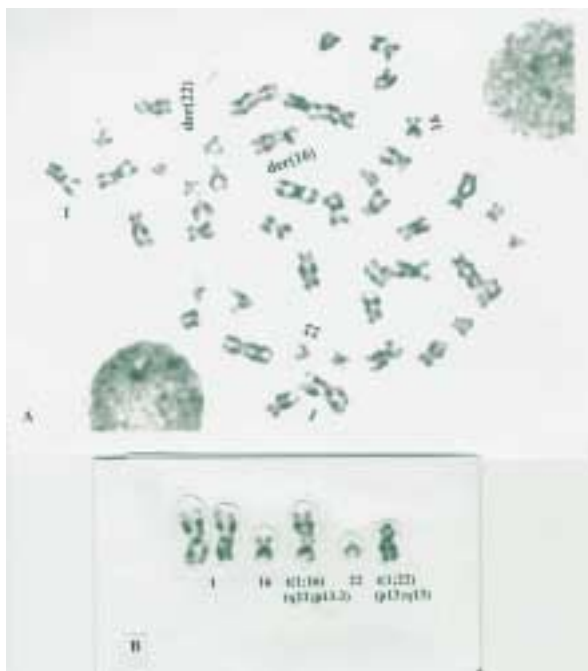


Fig. 13. Atypical cytogenetic abnormalities in MDS patients.

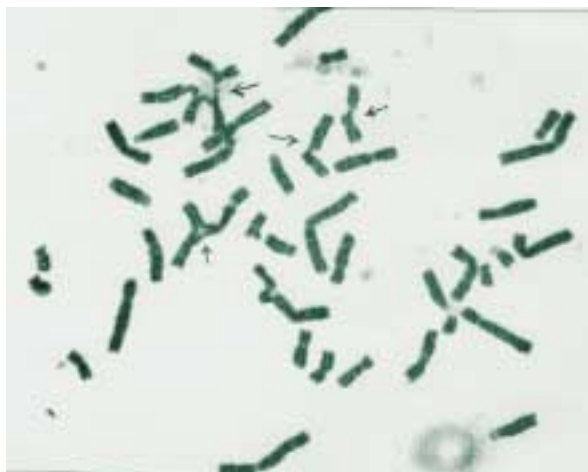


Fig. 14. Chromosomal breakage seen in MDS patients.

HAEMOGLOBINOPATHIES/BLOOD DISORDERS AMONG TRIBALS

Nutritional Anaemia and Haemoglobinopathies amongst Tribal Populations of India

A multicentric intervention programme on nutritional anaemia and hemoglobinopathies amongst some primitive tribal populations of India is ongoing in four states (Maharashtra, Gujarat, Orissa and Tamil Nadu).

A total of 11511 individuals from the primitive tribal groups of *Katkaris, Madias, Kotvadias, Kolchas, Irulas, Kurumbas, Molukurumbas, Paniyas, Bondos and Didayis* were clinically examined. Mild to moderate anaemia was found to be more common in all the tribes and the females were more anaemic than males. Iron deficiency anaemia was found to be very high among *Juangas* followed by *Didayis, Bondos, Irulas, Madias, Kurumbas, Katkaris, Paniyas, Kolchas Kolams, Kotvadias, Moolu kurumbas* and *Kathodis* (Fig.15) A higher percentage of sickle cell trait and homozygotes was found among the *Madias, Paniyas, Irulas, Kurumbas* and *Moolu Kurumba* tribal groups, whereas β -thalassemia trait was more common among the *Kolchas, Katkaris, Juangas, Didayis, Bondos* and *Kolams* (Fig. 16). G6PD deficiency was found in all the tribals. Iron deficiency anaemia was also found to be very common among the sickle cell anaemia, sickle cell trait and β -thalassemia trait cases. Prevalence of malaria was found to be high in cases with sickle cell trait, sickle homozygotes, β -thalassemia trait and G6PD deficiency. Hepatosplenomegaly was found to be common in AS, SS and β -thalassemia cases.

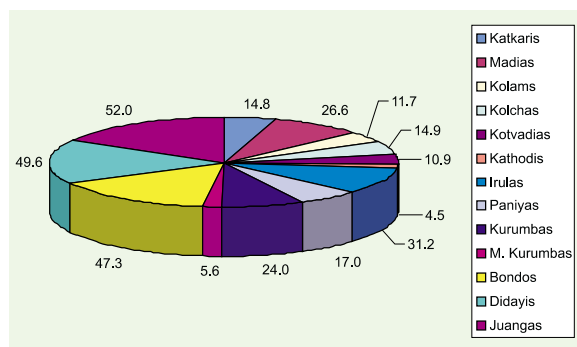


Fig. 15. Prevalence of iron deficiency anaemia in different tribal groups.

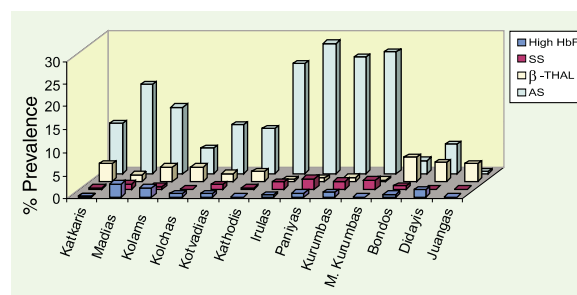


Fig. 16. Haemoglobinopathies in different tribal groups.

Basic Medical Sciences



Intervention and follow up is continuing in nutritional anaemia and sickle cell anaemia cases. Folic acid and Fersolate tablets have been given to cases of nutritional anaemia and sickle cell disease without iron deficiency. Sickle cell anaemia patients were advised to take electrical powder and plenty of fluid during vaso-occlusive crisis. An improvement in Hb and reduction in FEP levels was observed in cases taking tablets regularly. Hepatitis, *H. influenzae* and pneumococcus vaccines were given to sickle cell anaemia cases. Report Cards were distributed to all the individuals whose blood was tested. Genetic counselling was given to the unmarried young individuals to avoid marriage between carriers of haemoglobinopathies particularly Hbs and β -thalassemia and young carrier couples have been advised to come for prenatal diagnosis.

Haemoglobinopathies among Scheduled Castes and Scheduled Tribes of Madhya Pradesh

Genetic mapping of the haemoglobinopathies was done among the scheduled tribes (ST) and scheduled castes (SC) of Shahdol district by the RMRC, Jabalpur. Studies revealed that sickle haemoglobin is the main form of haemoglobinopathy in them with very high

prevalence in *Panika* tribe (28.6%) (Fig.17). Most of the sickle cell disease patients have mild to moderate anaemia and high level (10-15%) of fetal haemoglobin. Prevalence of anaemia is very high in *Gond* and *Baiga* tribes (70-80%) as compared to *Panikas* and *Chaudharys* (55%).

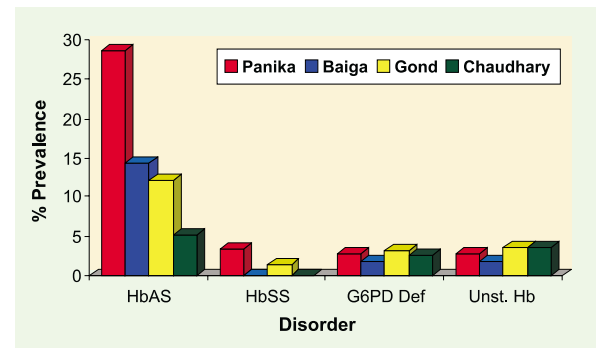


Fig. 17. Genetic disorders among STs and SCs in Shahdol district.

A semi-longitudinal study on sickle cell disease patients revealed that painful crisis, fever and abdominal/splenic pain are the common clinical manifestations. About 10% of patients had massive spleen (>9 cm), requiring multiple blood transfusions. Most (70-80%) of these patients *i.e.* aged upto 15 yr showed moderate to severe growth retardation.

