HIGHLIGHTS DOCUMENT

2008-09

INSTITUTE OF PATHOLOGY
(INDIAN COUNCIL OF MEDICAL RESEARCH)
NEW DELHI

EXECUTIVE SUMMARY

Institute of Pathology has made seminal contributions to various diseases covered under thrust areas with attempts to bridge basic studies to the clinic. The primary approach of scientists has been to understand genetic mechanism associated with virulence and drug resistance and to identify biomarkers for risk, prognosis and new therapeutic targets in various cancers and infectious diseases.

One breast cancer cell line (PCB20) has been established from primary breast cancer from an early onset breast cancer case (32 years) as an important tool to study molecular carcinogenesis involved in early onset breast cancer and pre-clinical drug trials. Studies on gene expression profile and promoter hypermethylation have been initiated in early onset breast cancer cases using micro-array technology to understand molecular pathogenesis. Correlation of expression of type 1 growth factor receptor genes EGFR, c-erbB-2, c-erbB-3, MDR1 and AR genes in locally advanced breast cancer cases with response to neo-adjuvant chemotherapy showed that AR gene carries independent predictive role for responders. Study to identify CAG micro-satellite repeats in androgen receptor gene, (TTTA) repeat analysis in CYP19 gene, polymorphism in prostate specific antigen (PSA) gene and MLH1 gene in prostate cancer (CaP) with correlation to genetic susceptibility and progression of carcinoma showed protective role of GG genotype of PSA gene, slight association of genotype A2A2 of CYP19 [TTTA] repeat and significant association of CC genotype at -93 position of the core promoter region of MLH1 gene with the risk of prostate carcinoma. Study on role of effectors function of cyclooxygenases (Cox-1 and Cox-2) and associated different cytokines in PBMCs in invasive and non-invasive TCC of urinary bladder showed increased Cox-2 expression in invasive cancer patients in comparison to non-invasive patients and normal healthy controls. Significant variation in IL-1β and IL-6 levels was observed in patients in comparison to normal group. The expression of CD74 was also found high in cancerous patients compared to controls.

Among studies on cancers in north-east region of India, investigation on genetic factors associated with tobacco and familial aggregation in esophageal carcinoma by micro-array has been completed. Pathways found significantly upregulated in both tobacco associated and familial cancers include MAPK pathway, neuroactive ligand receptor interaction, calcium signaling pathway and downregulated pathways include extra-cellular matrix organization, structural constituent of ribosome, cell communication and apoptosis activity. The familial esophageal cancers also showed upregulated B-cell receptor signaling pathway and downregulated genes involved in metabolism of xenobiotics and natural killer cell-mediated cytotoxicity. Validation of differential expression of subset of genes by QRT-PCR and tissue micro-array in familial and non-familial cases showed no significant difference in expression of these genes in two groups suggesting familial clustering occurs as result of sharing of common environmental factors. In tobacco associated cancers in north-east region, no significant contribution of GSTM1 and GSTT1 null polymorphisms was

found in oral and gastric cancers. Polymorphism in codon 72 of p53 gene showed that genotype pro/arg may act as a risk factor for gastric cancer while genotype pro/pro acts as protective factor for lung cancer. Gene expression studies have been initiated in oral, gastric and lung cancers and copy number analysis has been done in esophageal cancer using 10K array. In pesticide associated cancers, no significant contribution of mutations in BRCA1 & 2 genes, CYP17 gene, p53 gene codon 72 polymorphisms have been found to the risk of breast cancer; however GSTP1 null polymorphisms were found significantly associated with risk of breast cancer in this region. Copy number analysis by micro-array is being done to identify genes associated with risk and progression of breast cancer in this region. In non- Hodgkin lymphomas in north-east region, no significant association of bcl-IGH translocations and GST gene polymorphisms was found with the risk of this cancer.

Study on prevalence and prognostic value of FLT3 gene mutations in acute myeloid leukemia cases showed alterations in FLT3 gene in 23% patients; however preliminary results showed no significant difference in response to induction therapy between patients with or without FLT3/ITD mutation. Study on expression of activator and target genes of nuclear factor-kappa B (NF-kB) transcription factor in acute leukemia showed significant differences in expression level of IkB-α, IKK-B, P53, cIAP-2 and survivin in samples of AML and ALL. Significantly low expression of p53 was found in non-responder group of AML patients which correlated with IKK-alpha gene expression. Expression level of cIAP-2 was significantly lower in non-responder group of ALL patients. A high-throughput tissue micro-array (TMA) chip containing 300 brain tumors from archival paraffin blocks at IOP according to subtypes and histological grades based on WHO classification has been constructed using manual Tissue Arrayer with core diameter of 1.0 mm and used to study the protein expression of the differentially expressed genes identified by cDNA micro-array at National Cancer Institute for analysis of potential diagnostic and prognostic biomarkers using immunohistochemistry TMA-IHC.

Study on role of *Chlamydia* heat shock proteins in pathogenesis of genital tract infection in women showed that in cervical epithelial cells, cHSP60 and cHSP10 had a different pattern of expression in infertile women compared to fertile women reflecting probable difference in the metabolic state of *Chlamydia* with the presence of an abnormal cryptic form of *C. trachomatis* in infertile women. These results strongly support involvement of cHSP60 and cHSP10 in immunopathology of infertility. Study on the correlation of *Chlamydia* infection load with immune factors showed significantly higher inclusion counts in *Chlamydia*-positive fertile women compared to women with Fertility-Related Disorders (FD) with lower recovery of *Chlamydia* from the cervix. Further, *Chlamydia* IFUs correlated positively with CD8, pDC, IL-8, CRP and IFN-γ in women with MPC. In women with FD, *Chlamydia* IFUs correlated positively with pDC, IL-10 and estradiol and negatively with CD4 and IFN-γ levels. This data suggests that clinical condition presented is decided by interplay of infectious load and host immune responses. Significant decrease in levels of interleukin (IL)-8, interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α) was observed in cervical secretions of *Chlamydia*-positive

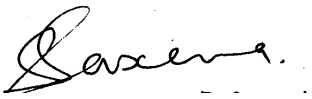
women with and without infertility problem after administration of azithromycin as compared to levels before therapy suggesting that azithromycin modulates the production of cytokines in process of eradication of infection. In the study on role of iron on pathogenesis of *Chlamydia trachomatis,* expression of transferrin receptor (TfR) was found down-regulated; whereas expression of ferritin heavy chain (FHC) was up-regulated in *C. trachomatis* (CT) infected HeLa 229 cells. Expression of TfR in infected cells did not change upon addition of iron cheater deferoxamine (DFX) and iron source ferric ammonium citrate. Expression of iron regulatory protein (IRP)-1 predominates over IRP-2 in infected cells. Attenuation in binding activity of IRP- IRE was observed in electrophoresis mobility shift assay of infected cells and is central to iron homeostasis. To study the role of inclusion membrane proteins (Incs) in *Chlamydia* pathogenesis, there is a need for understanding their role in host-pathogen interactions. Role of *Chlamydia pneumoniae* in Coronary Artery Disease (CAD) patients showed high seropositivity for *C. pneumoniae* specific IgG, IgA in CAD patients compared to healthy controls.

Identification of a novel ubiquitin-like system - a clone homologous to human Uba5 (Ubiquitin activating enzyme E1) in the protozoan parasite *Leishmania donovani* and detection of LdUba5 gene transcripts in infected bone marrow samples from leishmaniasis patients suggest their role in the disease pathogenesis in the human host. Transcriptome profiling for identification of antimony resistance determinants in *Leishmania donovani* isolated from Indian patients of Kala-azar showed genes coding for Protein Surface Antigen 2 (PSA2), Histone (H1), Histone 2A (H2A), Histone 4 (H4), MAP-kinase, and two hypothetical proteins transcribed more abundantly in the antimony resistant parasite in comparison to sensitive, while genes encoding amino acid transporter, conserved hypothetical protein with ATPase F1/V1/A1 complex signature and conserved hypothetical protein with Myb DNA binding signature showed consistent over-expression in sensitive parasites. *In vitro* susceptibility of isolates to antileishmanial drugs (Miltefosine, Amphotericin B, Paromomycin and Sitamaquine) significantly correlated with one another raising the possibility of cross- resistance. The data indicates that paromomycin may be a more effective treatment option as parasites from HR and LR region had similar susceptibility to it. Evaluation of host immuno-determinants involved in pathogenesis of Kala Azar and Post-Kala-Azar Dermal leishmaniasis using cDNA array implicates the presence of effecter (IFN-γ, TNF-α) and regulatory (IL-10, TGF-ß) molecules together with apoptosis (FasL /TRAIL) and chemokines related genes (MIP-1α, MIP-1α and MCP-1). The data implies that Th1/Th2 paradigm of resistance/susceptibility in humans against intracellular parasite *Leishmania* is an oversimplification of a complex network of effecter/regulatory interactions. Analyses of the intralesional cytokine gene expression in post-kala-azar dermal leishmaniasis (PKDL) and kala-azar (KA) patients revealed a significant down-regulation of TNFR1 transcript in both PKDL and KA compared with control. Investigation of matrix metalloproteinases, known to be induced by TNF-alpha, and the tissue inhibitors of matrix metalloproteinases (TIMPs) provided evidence for the roles of TIMP-1 and TIMP-3 in the pathogenesis of PKDL. Analysis of immune-determinants

in patients of cutaneous leishmaniasis caused by *L. Tropica* revealed IL-8 as an effecter immune-determinant in the pathogenesis of cutaneous leishmaniasis which may facilitate influx of polymorphonuclear cells at inflammatory site serving as parasite "shelter", while MCP-1 stimulates the parasite killing by macrophages via generation of nitric oxide. Studies on Multilocus Microsatellite Typing (Mlmt) reveals genetic homogeneity of *Leishmania donovani* strains in the Indian subcontinent including Bangladesh, Bihar (India) and Nepal which formed a very homogeneous population regardless of geographical origin, clinical manifestation, and whether they presented *in vitro* or *in vivo* susceptibility to antimonial drugs.

Studies on optimal attenuation conditions for 3T3 fibroblasts for use as feeder cells indicated that successful attenuation is dependent on numerical dosing with concomitant optimization in the stimulation of keratinocyte cell proliferation. Investigation into the utility of a patented synthetic thermo-reversible hydro gel polymer (TGP) as supportive matrix towards the development of 3-D composite skin showed that TGP perhaps specifically stimulates only those keratinocytes that have inherent stemness not differentiation. Studies on health hazards of phthalates vis-à-vis idiopathic male infertility showed a significant decline in testosterone level in infertile group. The decline showed positive correlation with increasing exposure to occupational phthalate. The testosterone levels also showed corresponding decline with sperm count and motility morphology.

During last two years the Institute has been undergone major building renovation activities occupying bulk time of scientists but the Highlights depict no compromise on scientific achievements in way of publishing papers in high impact journals and obtaining extramural funding from national and international agencies through stiff competition. Dr. Sunita Saxena received **K.C. Basu Mullik Award** for 2008 from Indian Association of Pathologists and Microbiologists and Dr. Poonam Salotra was elected **Fellow of the National Academy of Sciences, India**. Dr. AP Singh was awarded **Indo-US Research Fellowship** to work at Advanced Technology Center, Tissue Array Research Lab, National Cancer Institute, NIH. Many PhD students received best paper (oral/poster) awards in respective national conferences. The Institute continues to contribute significantly in human resource development by conducting various academic activities, viz.: DNB, Ph.D, summer fellowships and WHO training programs. The sincere efforts of our scientists, technical and administrative staff in enhancing infrastructure and scientific activities of Institute deserve my sincere thanks and appreciation.



(Dr. Sunita Saxena)
Director

TUMOR BIOLOGY

I. BREAST CANCER

1. Study on Gene Expression and Hypermethylation Profiles in Early Onset Breast Cancer

Breast cancer diagnosed at young age (< 45 years) shows clinically different characteristics compared to breast cancers diagnosed at older ages. Younger patients more frequently exhibit aggressive features such as large tumor size, high histological grade, positive lymph nodes, absence of steroid receptors and high S-phase fraction suggesting young age itself to be an independent predictor of adverse prognosis. Thus early-onset breast cancer may be biologically different from breast cancer in older patients. This study has been undertaken to analyze differential gene expression profile and role of promoter hypermethylation in early onset breast cancer cases using micro array technology in non familial and non-BRCA mutant patients.

Gene expression profiling of 10 primary breast cancers were carried out using illumina bead array chips. Universal Human Reference RNA (from *Stratgene*) was used as control. About 1800 differentially expressed genes were obtained, however detailed analysis will be done after carrying out gene expression analysis in greater number of samples.

2. Establishment and Characterisation of Cell Lines from Primary Breast Cancers

Cell lines are the most useful tools for studying cancer biology and development of new therapeutic strategies. This study aims to develop and characterize cell lines from primary breast tumours especially from early onset cases (<45 years). During the year under report, 31 breast tumours were collected and used for initiation of primary cultures. Twelve primary cultures have been initiated during this year, together 19 primary cultures established in first and second year. All the primary cultures established were subjected to purification using differential trypsinisation, cloning and Magnetic Activated

Cell Sorting (MACS) using EpCam and cytokeratin antibodies. These purified cultures were characterized for expression of epithelial markers (cytokeratins and epithelial membrane antigen), mesenchymal marker (vimentin) and biological markers (Estrogens receptor, Progestron receptor, Her 2 neu). One breast cancer cell line (PCB20) has been established during the year under report from an early onset breast cancer case (32 years). This cell line (PCB 20) has shown positivity for pancytokeratins and EMA, a faint positivity for vimentin and negative expression for estrogen receptor, progestron receptor and Her 2 neu (Fig. 1).

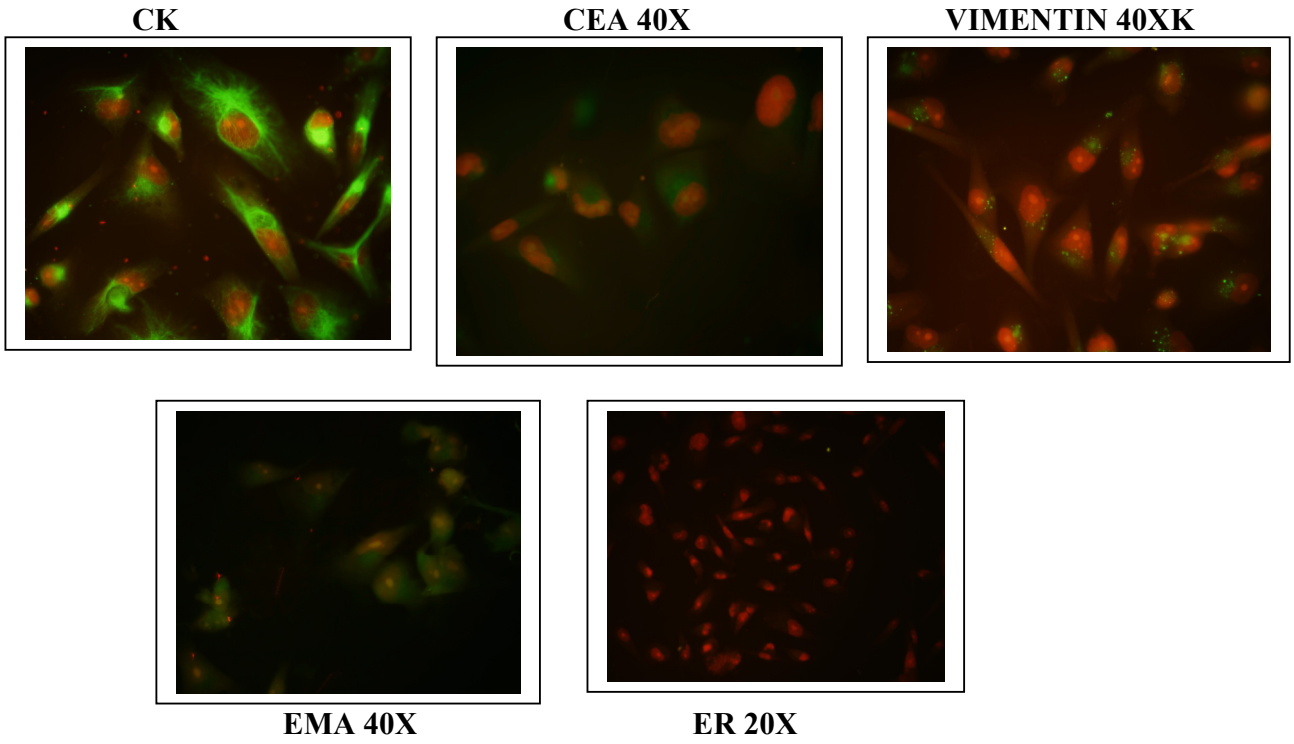


Fig. 1: Expression of Biological Markers in PCB 20 Cell Line

3. Type 1 Growth Factor Receptor Family : Expression And Correlation With Response To Neo-Adjuvant Chemotherapy In Locally Advanced Breast Cancer

Despite tremendous improvement in treatment of locally advanced breast cancer, the prognosis remains dismal in many patients. The aim of this project is to analyze the expression of type 1 growth factor receptor genes EGFR, c-erbB-2, c-erbB-3 along with MDR1 and AR genes in locally advanced breast cancer cases with correlation to response to neo-adjuvant chemotherapy.

Thirty-eight matched samples of pre- and post-NACT tumor tissues were collected during the year under report. Total RNA (TRIzol method) was isolated and cDNA was generated using High Capacity cDNA Archive kit (*Applied Biosystems*) according to the manufacturer's protocols.

The relative quantitation of expression levels of EGFR, c-erbB-3 and MDR1 genes in pre- and post-NACT breast tissue with human normal breast total RNA(*Ambion*) as a control sample was carried out by real time RT-PCR (*ABI 7000 SDS, Applied Biosystems*) with cDNA as template using TaqMan probe Assay. Clinically, 24 out of 38 patients were responders and 14 were non-responders. 16 out of 38 were pre-menopausal and 22 were post-menopausal. Relative quantization expression of EGFR, c-erbB-3 and MDR1 genes was also done.

The expression of EGFR and c-erbB-3 genes showed no significant difference in pre- NACT samples between responders and non-responders, however they were found significantly high in post-NACT samples in non-responder group compared to responder group of patients ($p=0.003$ and $p=0.036$, respectively). In non-responder group of patients, there was higher expression of EGFR ($p = 0.034$) and c-erbB-3($p=0.048$) in post-NACT samples compared to pre-NACT samples. On the contrary, in responder group of patients, no significant difference was found in expression of EGFR and c-erbB-3 between pre- and post-NACT samples. However, expression of MDR1 ($p= 0.017$) gene in post-NACT samples in responder group of patient was found significantly high compared to pre-NACT samples. These results suggest that EGFR and c-erbB-3 do not carry predictive value for response to neo-adjuvant chemotherapy .

Based on the above results and previous studies, we would like to include some AR associated genes like TGFB, SMAD3, PSA, ARA70 in our study, since we have already reported that AR gene may be a good predictor for response to neo-adjuvant chemotherapy in locally advanced breast cancer to understand the mechanism involved.

II UROGENITAL MALIGNANCIES

1. Micro-satellite Instability In Androgen Receptor Gene And P53 Polymorphism In Prostate Carcinoma In Indian Males

The goal of the present study is to identify CAG micro-satellite repeats in androgen receptor gene, (TTTA) repeat analysis in CYP19 gene and p53 polymorphisms/ mutations in prostate cancer and their correlation with genetic susceptibility and progression of carcinoma. It also aims to identify noble polymorphisms and mutations in MLH1 and MSH2 genes, expression profile of Mismatch Repair System genes associated with prostate cancer. The study also includes examining the impact of genetic polymorphism in prostate specific antigen (PSA) gene on the risk for prostate cancer.

Blood samples from 106 cancer prostate cases, 120 BPH cases and 106 normal healthy controls were collected. DNA from all the samples was extracted and analyzed for G>A polymorphism in *PSA* gene, CYP19 [TTTA] repeat analysis and C/T transition in MLH1 gene.

Genotypic analysis of allelic frequencies of PSA polymorphism at positions –158 G>A polymorphism showed that the GG genotype plays a protective role against the risk of prostate cancer with OR 0.26 (95% CI, 0.81- 0.86, p=0.03) when compared with CaP and controls. The CYP19 gene encodes aromatase enzyme that converts androstenedione/testosterone to estrone/estradiol, has been proposed to be associated with CaP risk. The genotypic analysis of *CYP19* [TTTA] repeat by Genescan analysis showed slight association of genotype A2A2 with CaP cases while heterozygous A1A2 was predominant in controls. The genotypes were confirmed by sequencing (Fig. 1)

A C/T transition at -93 position of the core promoter region of MLH1 gene was also identified and evaluated using SNPshot genotyping methodology. We investigated and found that CC genotype increases the risk of prostate carcinoma as significant differences in the distribution of -93 C/T polymorphism between the CaP cases and controls (OR= 1.961, 95% CI, 1/023-3.760, p= 0.05), BPH and controls (OR= 2.039, 95% CI, 1.078-3.857, p= 0.039) were noticed.

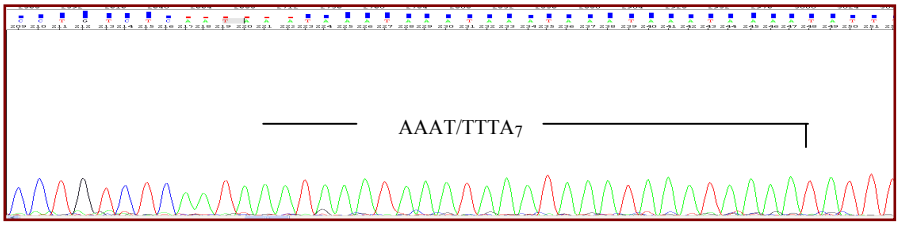


Fig. 1 : Electrogram showing the CYP19 [TTTA] repeat in one of the CaP case with the repeat length of 7.

2. Study On The Role Of Cyclooxygenases And Cytokines Dysfunction In Invasive And Non Invasive Transitional Cell Carcinoma Of Urinary Bladder

Cyclooxygenase derived prostaglandins contribute to tumor cell resistance to apoptosis, cell proliferation, angiogenesis and tumor progression. In the present study, we evaluated the role of cyclooxygenases (Cox-1 and Cox-2) and associated different cytokines in PBMCs to understand the role of cyclooxygenase in modulating the role of PBMC cytokines and their effector function in invasive and non-invasive TCC of urinary bladder.

Heparinized blood samples were collected from ten normal healthy individuals and 46 TCC patients from Urology Department, Safdarjung Hospital and studied for expression of cox-1 and cox-2 with correlation to cytokines by flow cytometer. Biopsy samples from tumors and non-tumorous part of bladder were also collected from same patients to study the expression of cox-1 and cox-2 using immunoflurorescence and IHC. Out of 46 cases, 6 cases were of invasive TCC. Out of these six invasive cases, 2 cases were from grade II and 4 were from grade III.

In non-tumorous bladder, urothelium Cox-2 expression was nil while Cox-1 expression was observed in 70% cases. On the other hand in tumor tissue, Cox-2 expression was not seen in low grade tumors of both invasive and non-invasive cases while expression increased from 50-70% and from 60-100% from grade II to grade III tumors both in non-invasive and invasive cases. Cox-1 expression showed uniform distribution from low grade to high grade tumors of non-invasive group. In invasive group, expression of Cox-1 decreased from low to high grade tumors.

The flow cytometric analysis of cytokine expression on PBMCs showed the higher expression of IL-1β in patients in comparison to healthy individuals. The mean percentage of double positive cells for IL-1β with Cox-2 and TNF with Cox-2 incresead to 38.6±7.28 and 36.51± 4.46 from 22.37±13.01 and 22.63±11.92 respectively in cancer patients in comparison to normal healthy groups. Cox-2 expression was found increased in cancer patients in comparison to normal healthy individuals (Fig.1). Cox-2 expression was seen in 4 out of 5 invasive TCC samples. Significant variation in IL-1β ($p>0.004$) and IL-6 ($p>0.01$) level was observed in patients in comparison to normal group (Fig.1). The expression of CD74 was also found high in cancerous patients ($p>0.002$) compared to controls.

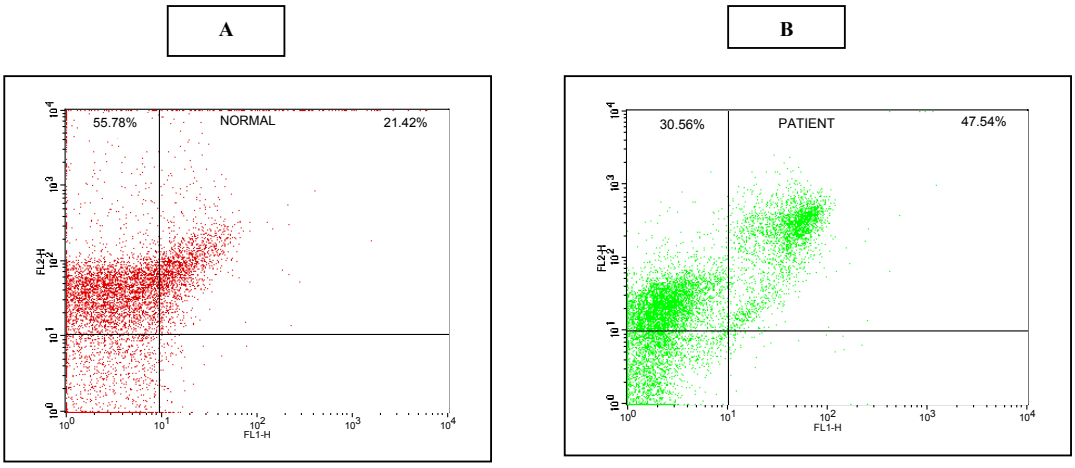


Fig. 1: Dot Plots are showing the expression of Cox-2 and IL-6 in PBMCs in Normal Healthy Individual (A) and in TCC patients (B) using Flow cytometer.

III. CANCER IN NORTH-EAST REGION OF INDIA

1. Role Of Tobacco Use In Causation Of Cancer In North East India
(ICMR Multi-Centric Task Force Project, 2005-09)

This study has been undertaken to investigate the role of tobacco consumption for high incidence of oral, lung, stomach and esophageal cancers.

Oral cancer

Genotyping of GSTT1 and GSTM1 Genes:

A multiplex PCR method was used to detect the presence or absence of the GSTT1 and GSTM1 genes in the genomic DNA samples of patients and age and sex matched healthy controls. β-globin was used as an internal control. The absence of 480bp band indicates GSTT1 null and the absence of 215bp indicates GSTM1 null genotypes.

One hundred and thirty six cases with oral cancer and 270 age and sex matched controls were included in the study. The frequency of GSTM1 null genotype was found to be 48 % in oral cancer cases and 45% in controls. The frequency of GSTT1 null genotype was found to be 30% and 31% in cases and controls respectively. There was no significant association of GSTM1 and GSTT1 null genotypes between cases and controls. Thus results do not support the hypothesis that GSTM1 and GSTT1 null genotypes increased the risk of oral cancer.

Polymorphism in codon 72 of p53 gene:

This gene contains single nucleotide polymorphism that encodes either arginine (Arg) or proline (Pro) at amino acid codon 72 of the p53 protein. The allele constitution at codon 72 of the tumor suppressor gene p53 plays a major role in inducing apoptosis in p53 mutant cells. Polymorphic variant of p53 at codon 72, has been found to be associated with cancer susceptibility, but no data is available on its role in oral cancer in high risk northeastern population of India.

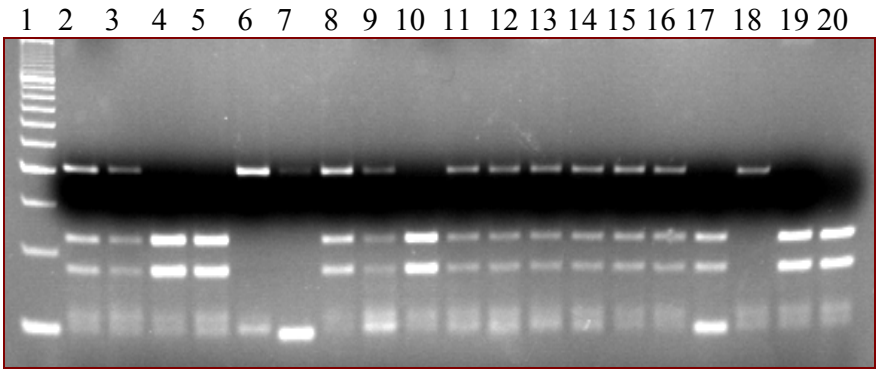


Fig. 1: Analysis of RFLP digested PCR products of codon 72 of p53 gene by agarose gel electrophoresis (3.5%).
Lane 1, 50bp ladder.
Lane 2,3,8,9,11,12,13,14,15 and 16, Arg/Pro (heterozygous)
Lane 4,5,10,17,19 and 20, Arg/Arg (homozygous)allele,
Lane 6,7 and 18, Pro/Pro (homozygous) allele.

One hundred and ten oral cancer cases and 175 age and sex matched controls were included in the study. The frequencies of pro/pro, pro/arg and arg/arg genotypes in cases were 20%, 55% and 25% respectively whereas the frequencies of pro/pro, pro/arg and arg/arg genotypes in controls were 22%, 54% and 24% respectively (Fig.1).

No significant difference was found in different genotypes Pro/Pro, Pro/Arg and Arg/Arg in oral cancer patients when compared with controls.

CYP1A1 Polymorphism:

This enzyme is responsible for the metabolic activation of most of the carcinogenic PAHs like benzo(a)pyrene from tobacco smoke. The oxygenated and metabolically active products are further metabolized to more polar and water soluble products by Phase II enzymes that are easily excreted from the body. Some of the initial metabolites are highly reactive oxygen species that can damage DNA and potentially promote cancer development.

Polymorphisms in the CYP1A1 gene were detected by PCR-RFLP using the MSP1 restriction endonuclease. Restriction digested fragments were analysed in 3.5% agarose gel.

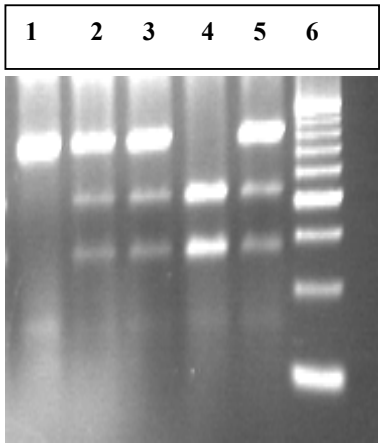


Fig. 2: RFLP digested fragments for Cy1A, polymorphism
Wild type (W/W)- single band at 340bp eg. Lane 1
Homozygous variant allele (V/V)- two bands at 200 and 140 bp eg. Lane4
Heterozygous Variant allele (W/V) – all three bands present eg. Lane 2,3,5
Lane 6, 50bp ladder

The frequency of wild type (W/W) genotypes was 38% in cases and 42% in controls. Variant genotypes (W/V or V/V) frequency was 62% in cases and 58% in controls (Fig.2). Preliminary results showed no significant difference in the frequency of polymorphisms of the CYP1A1 gene in cases and controls suggesting that there may be no association of the CYP1A1 polymorphism with oral cancer in northeast Indian population.

Gene expression study:

Gene expression study using oligonucleotide chips on five samples obtained from tumor tissue and adjacent normal tissue from oral cancer patients showed involvement of various biological processes and pathways that are differentially expressed including

- Toll-like receptor signaling pathway,
- Focal adhesion
- Apoptosis
- Cytokine-cytokine receptor interaction
- MAPK signaling pathway Significantly down regulated pathways were:
- Genes involved in neuroactive ligand-receptor interaction
- Wnt signaling pathway

Gastric cancer

Genotyping of GSTT1 and GSTM1 Genes:

One hundred and thirty three cases with gastric cancer and 270 age and sex matched controls were studied for genotyping of GSTT1 and GSTM1 genes. The frequency of GSTM1 null genotype was found to be 36.84 % in gastric cancer cases and 44.81 % in controls. The frequency of GSTT1 null genotype was found to be 36.54 % and 31.48 % in cases and controls respectively. GSTM1 and GSTT1 null genotypes were not significantly different in cases and controls and may not be associated with gastric cancer risk.

Polymorphism in codon 72 of p53 gene:

One hundred and ten gastric cancer and 175 age and sex matched controls were studied for polymorphisms in codon 72 of p53 gene. The frequencies of pro/pro, pro/arg and arg/arg genotypes in cases were 13%, 67% and 20% respectively whereas the frequencies of pro/pro, pro/arg and arg/arg genotypes in controls were 22%, 54% and 24% respectively. The frequency of pro/arg genotype was significantly higher in cases (p=0.03, OR=1.73) as compared to controls. Preliminary results suggest that pro/arg genotype may act as a risk factor for gastric cancer.

Detection of *H. pylori* in tumor tissue:

DNA was extracted from 10 tumor tissues collected in PBS using HiPurA DNA Extraction Kit [*HiMedia*].

Standardization of PCR for Ure A gene for detection of *H.pylori* in the tumor tissue was done using published primer sequences (Fig. 3).

Primer sequences for Ure A gene

Gene	Primer sequence
Ure A	**Forward primer** 5'-GCC AAT GGT AAA TTA GTT-3' **Reverse primer** 5'-CTC CTT AAT TGT TTT TAC-3'

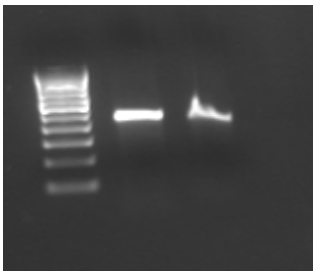


Fig 3:-1.5 % Gel showing the PCR product of ure A gene

Lung Cancer
EPHX1 polymorphism:

Xenobiotic-metabolizing *mEH*3, catalyzes the hydrolysis of arene, alkenes, and aliphatic epoxides to less reactive and more water soluble dihydrodiols through the *trans* addition of water. Two known polymorphisms affect enzyme activity in the *mEH* gene

- Substitution of histidine for tyrosine at amino acid position 113 (*Exon3*, Lower enzyme activity)
- Substitution of arginine for histidine at position 139 (*Exon4*, Increased enzyme activity)

The PCR conditions have been standardized and polymorphism is detected by PCR-RFLP method by using EcoRV and RsaI restriction enzymes for exon3 and exon 4 respectively (Figs. 4-5).

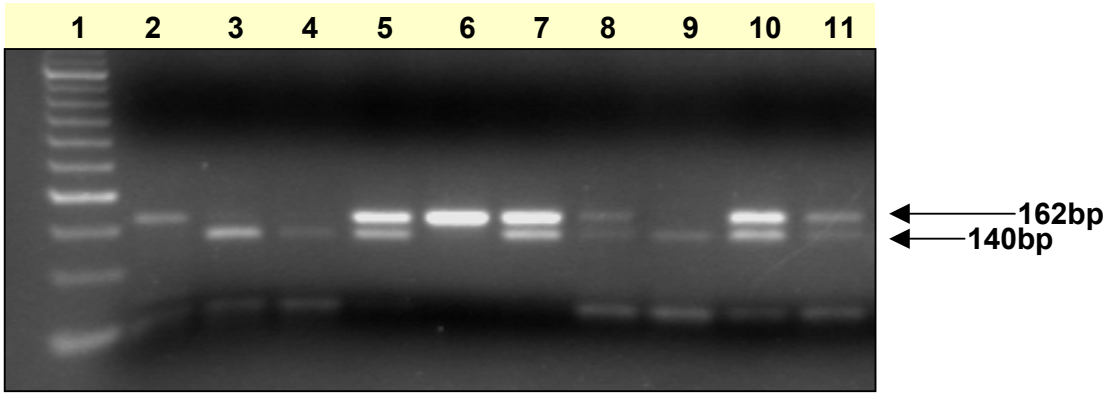


Fig 4: PCR-RFLP for Exon 3

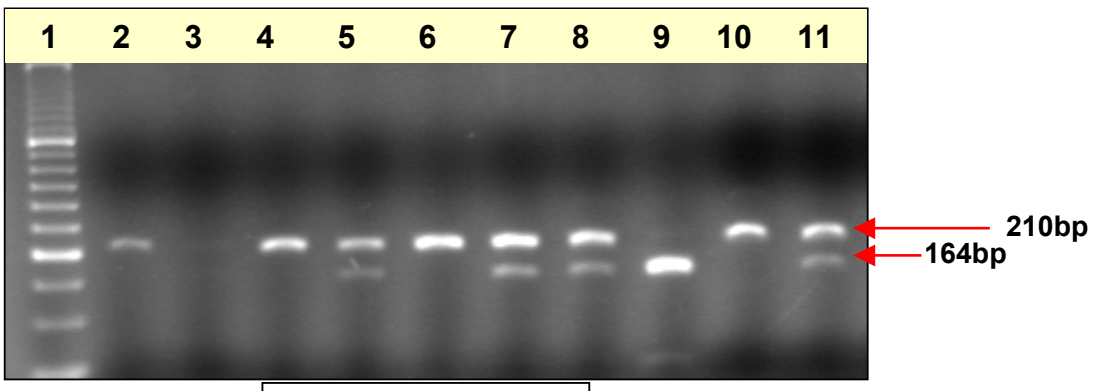


Fig 5: PCR-RFLP for Exon 4

TP53 Codon 72 Polymorphism :

Polymorphic variants of the p53 tumor-suppressor gene produce a guanine–cytosine change (G/C) at codon 72 resulting in arginine–proline (Arg[Pro) amino acid substitution. The functional impact of this p53 polymorphism has been reported and the Arg/Arg genotype seems to induce apoptosis with faster kinetics and to suppress transformation more efficiently than the Pro/Pro genotype. It is controversial if p53 cd72 polymorphism is associated with lung cancer risk.

Frequency of Pro/Pro genotype was higher in controls as compared to cases and it conferred a 40% less chance of developing the disease. The TP53 72P allele was found more often in controls than in lung cancer patients and carriers of the 72P allele had a reduced risk of the cancer. The well-documented polymorphism of TP53 gene may be a genetic risk factor for lung cancer susceptibility in Indian population.

Gene expression profiling:

Genes involved in protein binding, fibroblast growth factor receptor activity, structural constituent of ribosome, transcription co-activator activity, extra cellular matrix structural constituent and DNA binding were up-regulated whereas genes involved in regulation of wound healing and cell-cell adhesion were down-regulated in the tumor tissue.

Esophageal Cancer

Chromosomal Copy number Alteration :

Analysis of genome-wide chromosomal changes and copy number alterations (CNA) in esophageal carcinoma was done in 12 samples using high throughput method. Genomic DNA was isolated from endoscopic biopsy and blood samples obtained from the same patients with esophageal squamous cell carcinoma. Briefly, 250 ng of germ-line and tumor DNA was digested with XbaI, ligated to an Adaptor Xba fragment and amplified with a PCR primer complementary to the Adaptor Xba fragment. Purified PCR products were fragmented with Dnase and end-labeled by biotinylated-ddATP in the presence of Terminal Transferase (TdT). The labeled DNA was hybridized to the 10K

chip and stained sequentially with Streptavidin, biotinylated antistreptavidin and streptavidin-R-phycoerythrin conjugates. The chips were scanned and hybridization signals was detected by Affymetrix Microarray Suite 5.0 software. Hybridization and detection were done with an Affymetrix Fluidics Station 450 and GeneChip Scanner 3000. Genotype calls was generated using the Genotyping Tools software. Chromosome Copy Number Analysis Tool 2.0 software (CNAT) from Affymetrix was used for further analysis. Each array allows analysis of 11,555 SNPs, distributed evenly across the genome with a mean interval of 105 kb and median interval of 210 kb. Each SNP on the array is represented by 40 different 25-bp oligonucleotides, each with slight variations that allow accurate genotyping. Hybridization to each probe was assessed using a GeneChip Scanner (*Affymetrix*) and results scored using proprietary software (*GDAS, Affymetrix*). GDAS Mapping Algorithm uses a model-based approach to do allele calling for all SNPs on GeneChip 10K mapping arrays. Information about the linear chromosome location and upstream and downstream associated microsatellite markers and genes for each SNP was extracted directly from NetAffx Analysis Center7.

Allelic imbalances were found on chromosomes arms 1p36.13, 1q21.1, 2p14, 3q28, 3q27, 3q26.1, 5p15.2, 5q11.2, 6p25.3, 7q11.21, 9q31.3, and 17p13.1. These findings suggest that the gains and losses of chromosomal regions may contain ESCC-related oncogenes and tumor suppressor genes and provide important theoretic information for identifying and cloning novel ESCC-related oncogenes and tumor suppressor genes (Fig.6).

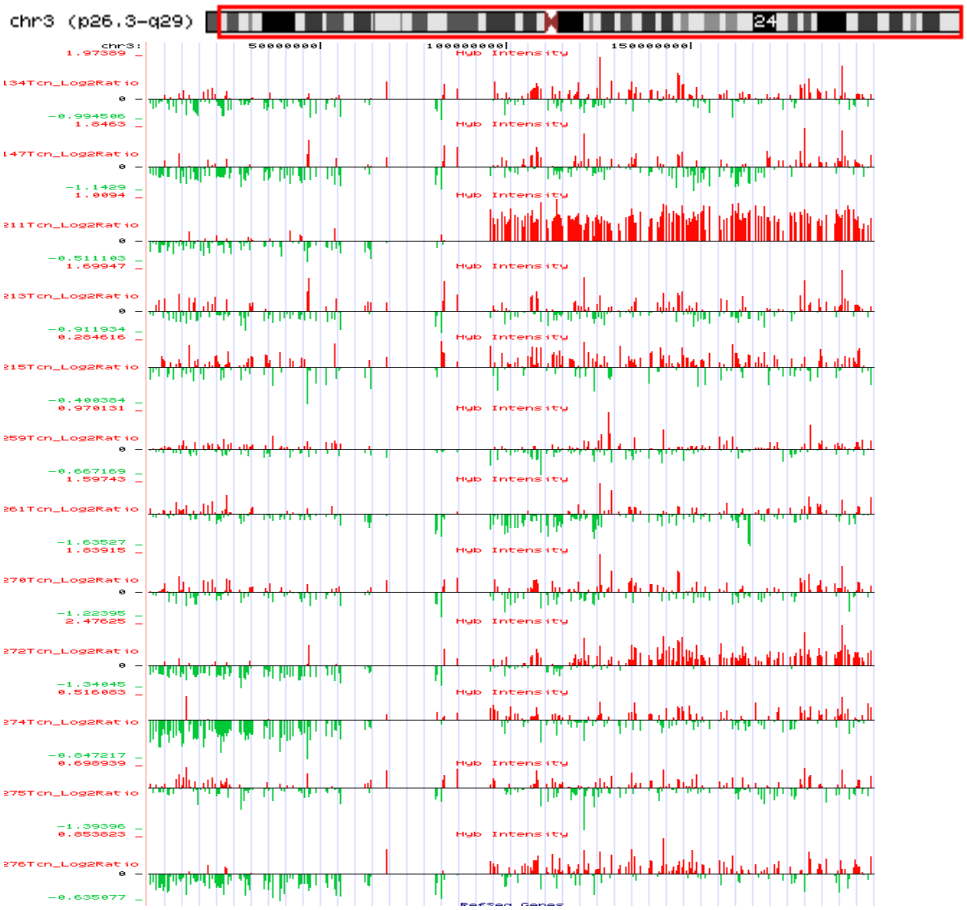


Fig. 6 : Copy number alteration profile of Chromosome 3 in all tumor samples

2. EFFECT OF PESTICIDE EXPOSURE IN CAUSATION OF CANCER IN NORTH EAST INDIA (ICMR Multicentric Task Force Project, 2005-09)

Breast Cancer

High incidence of breast cancer has been reported in various ethnic groups in north-east region suggesting role of both genetic and environmental factors. Apart from the high penetrance genes, there may be other genes that also increase the susceptibility to breast cancer. Candidates are proto-oncogenes and genes involved in metabolic, estrogen pathways.

CYP17 gene, encoding cytochrome P-450c17α enzyme, converts androgens to estrone and estradiol. Estrogen metabolism generates genotoxic intermediates leading to

DNA damage and breast carcinogenesis. The glutathione-S- tranferases (GSTM1, GSTT1) are involved in inactivation of these genotoxic intermediates. Polymorphisms in these enzymes are associated with less effective detoxification of potential carcinogens thus conferring increased susceptibility to breast cancer.

CYP17 Polymorphism:

5' UTR T>C polymorphism was analyzed in CYP17 gene by RFLP using MspA1 restriction enzyme in 117 breast cancer cases and 205 controls. A heterozygous (A_1A_2) status was seen in 49.7 % cases, A_2A_2 in 22.2% cases and A_1A_1 in 28.2% cases whereas a heterozygous (A_1A_2) status was seen in 59% controls, A_2A_2 in 16% controls and A_1A_1 in 24.82% controls. Although, the A2A2 genotype had a risk (O.R =1.4; 95%CI= 0.8394 to 2.6421), it was not significant. Therefore, Cyp17 T>C polymorphism does not appear to contribute to the risk of breast cancer in this population.

GST Polymorphism:

GST M1, T1 null and GSTP1 polymorphisms were analyzed using multiplex PCR and RFLP methodology in 117 cases and 205 controls. The GST T1 null polymorphism was seen in 28.2% cases as compared to 37.07% controls and GST M1 null polymorphism was seen in 19.65% cases as compared to 28.7% controls. The AA allele of GSTP1 was predominant in controls(61.9%) than in cases (41%). The GST T1 and M1 null genotype was not found to be a risk for developing breast cancer. The GG genotype of GSTP1 gene was a risk (O.R =3.19; 95%CI= 0.915 to 11.16) suggesting association of GST P1 with breast cancer risk.

TP53 Codon 72 Polymorphism:

One of the most well studied *TP53* gene polymorphism is *Arg72Pro*, located in codon 72 on exon 4, leading to arginine-proline substitution, which in turn results in structural alteration of the protein. p53 codon 72 polymorphism was analyzed in 117 breast cancer cases and 185 age matched controls by using RFLP using Bsh1236I restriction enzyme. A heterozygous (Arg/Pro) status was seen in 55 cases (47%), (Pro/Pro) in 34 cases (29%) and (Arg/Arg) in 28 cases (23.9%) whereas a heterozygous

(Arg/Pro) status was seen in 104 controls (50.7%), (Pro/Pro) in 52 controls (25.3%) and (Arg/Arg) in 28controls (23.9%). The Pro/Pro genotype was thus found more often in patients with breast cancer although results were insignificant.

Analysis of DNA Copy number variation in breast cancer:

DNA Copy number alterations can cause gene dosage, gene interruption, generation of a fusion gene, position effects, unmasking of recessive coding region mutations (SNPs in coding DNA) or other functional SNPs. Widespread DNA copy number alteration can lead directly to global deregulation of gene expression, which may contribute to the development or progression of cancer. Copy number changes may often encompass genes that may have important roles both in cancer susceptibility and drug response. Differences in the DNA sequence of our genomes influence most traits including susceptibility to disease. High throughput methods are useful in detecting submicroscopic rearrangements not visible by routine chromosome analysis.

Preliminary result showed copy number alterations in chromosomal regions corresponding to loci 1p35.3, 16p12.3, 10p14, 16q12.1 16q12.2, 8q24.128q21.13, 1q41 3q26.2 and 9q21.33, 5q31.1, 15q22.31, 14q32.32, 4q53.1, 4q25, 1p35.3 and 3p24.3 loci. These regions include genes like FHIT and p16.

IV. HEMATOPOIETIC-LYMPHOID MALIGNANCIES

1. Prevalence And Prognostic Value Of Flt3 Mutations In Acute Myeloid Leukemia

Genes involved in signal transduction have been the main focus of molecular analyses. Fms-like tyrosine kinase-3 (FLT3) is a class III receptor tyrosine kinase along with KIT, FMS and PDGFR, located on chromosome arm 13q12. The most common mutation in the FLT3 gene is internal tandem duplication (FLT3/ITD) of the region coding for the juxtamembrane (JM) domain of the FLT3 receptor. Another mutation reported is miss sense mutations in the tyrosine kinase domain (TKD) of the FLT3 gene. The most frequent are point mutation and deletions of codons 835/836 in second TKD of

the gene. Mutations of the FLT3 have a worse outcome and response to standard chemotherapeutic interventions.

Polymerase chain reaction (PCR) was performed using exon14 and exon15 specific primers. PCR products were analyzed on 3% agarose gels stained with ethidium bromide. Samples showing longer PCR products were considered as FLT3/ITDs and were confirmed by sequencing. FLT3/D835 mutations were detected by PCR-RFLP method by using EcoRV restriction enzymes (Fig. 1).

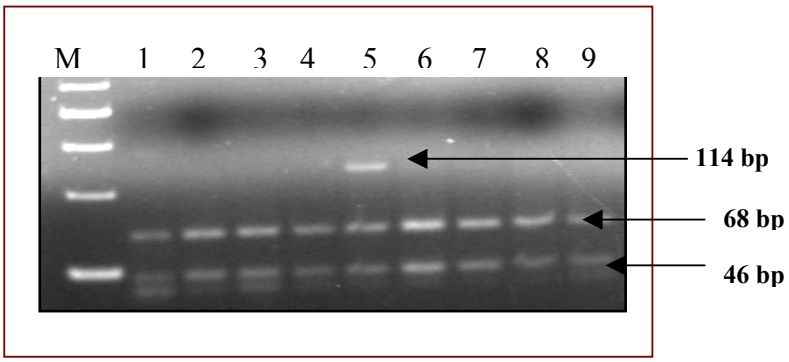


Fig. 1: Detection of D835 mutations in the FLT3 gene. Samples in lane 1-9 (except lane 5) show wild type FLT3. The digestion of amplified products of wild type FLT3 with EcoRV result in appearance of two bands i.e. 68 bp and 46 bp size. Lane 5 shows the undigested bands (114 bp) indicating mutation in FLT3 gene i.e. D835 mutation

133 cases of *de novo* AML with normal karyotype were included in the study. Alterations in FLT3 gene were detected in 23% patients. These aberrations include FLT3/ITD in 20% cases and D835 mutation in 43% cases which was significantly lower than that of ITD (P<.001). In 19% cases with ITD mutation, more than one duplication was detected. In 7.4% cases only one mutant band indicating either homozygous mutation of the allele or the loss of wild type allele for the FLT3/ITD was observed. None of the samples had a combination of FLT3/ITD and D835 mutation in the FLT3 gene. Preliminary results showed no significant difference in response to induction therapy between patients with or without FLT3/ITD mutation.

2. Expression Of Activator And Target Genes Of Nuclear Factor-Kappa B (NF-kB) Transcription Factor In Acute Leukemia

NF-κB pathway is a key mediator of genes involved in the control of cellular proliferation and apoptosis. Abnormalities in regulation are frequently seen in leukemia, however, activation is not uniform among AL patients. The aim of our study is to find specific regions in the NF-kB pathway that are activated/deregulated in patients with acute leukemia.

Thirty-five samples have been analyzed so far (20 AML and 15 ALL). NF-kB pathway was considered "activated" when the expression level of IK-B gene was 2.0 fold (cut-off) higher than the normal bone marrow sample. Gene Expression in AML and ALL were compared and significant differences in expression level of IkB-α, IKK-B, P53, cIAP-2 and survivin in samples of AML and ALL were found. Gene expression in responders and non-responders showed expression level of p53 to be significantly lower in non-responder group of AML patients. Expression level of cIAP-2 was significantly lower in non-responder group of ALL patients. Low expression of p53 was correlated with IKK-alpha gene expression in non-responders of AML. Since p53 regulates NF-kB pathway through the repression of IKK-α, loss of p53 function leading to transcriptional induction of IKK-α may be responsible for NF-kB mediated gene expression in ALL. Expression of IKK-α gene was found to be correlated inversely with the expression of p53 (correlation coefficient (r) = -0.9).

Expression of genes in NF-kB pathway was compared in CD34+ and. CD34- acute leukemia. In ALL, expression level of IkB-α was not found to be significantly different in CD34+ blasts (2.1±0.4 fold) versus CD34- blasts (2.4±0.4 fold), p=0.4. In AML, expression level of IkB-α was found to be higher in CD34- blasts (17.4±13.4 fold) compared to CD34+ blasts (4.7±3.07 fold) (p=0.1). In both ALL & AML, expression of cIAP-2 was higher in CD34- blasts while Expression of Bcl-2 was lower. More samples are being analyzed for calculating statistical significance of our results.

3. Identification Of Diagnostic And Prognostic Biomarkers In Brain Tumors: A Tissue Micro-Array Based Approach

Tumors of the central nervous system are one of the leading causes of cancer morbidity and mortality and remain difficult to cure despite advances in surgery and adjuvant therapy. Unfortunately, apart from routine histological classifications and WHO grading system, our ability to effectively stratify individual tumors into prognostically significant groups has yielded limited results. The current challenge in brain tumor research is to move from purely morphological classification to one that is based on genetic and molecular criteria. Several genetic aberrations and gene expression changes have been shown to occur during malignant transformation, development and progression of brain tumors. Identification, characterization and cataloging of these genetic alterations that correlate with the clinical behavior of these tumors has potential and needs to be investigated to establish the comprehensive molecular fingerprints of these tumors.

A high-throughput tissue microarray (TMA) has revolutionized the histopathological research. TMA is advantageous in comparison to standard histology sections as hundreds of tissue samples can be analyzed in a single experiment using 0.6-2.0 mm cylinders of tissue. It is amenable to a wide range of techniques including, immunohistochemistry and provides a judicious use of precious tissue, gives experimental uniformity, and analyzing a large number of samples improves statistical precision in addition to enhanced speed and quality of analysis.

The aim of the study was to construct tissue microarray chip containing 300 brain tumors from archival paraffin blocks according to subtypes and histological grades based on WHO Classification using manual Tissue arrayer with core diameter of 1.0 mm (Fig. 1) and study the protein expression of the differentially expressed genes identified by cDNA microarray at National Cancer Institute using immunohistochemistry TMA-IHC and study a comprehensive panel of target antigens for analysis of potential diagnostic and prognostic biomarkers.

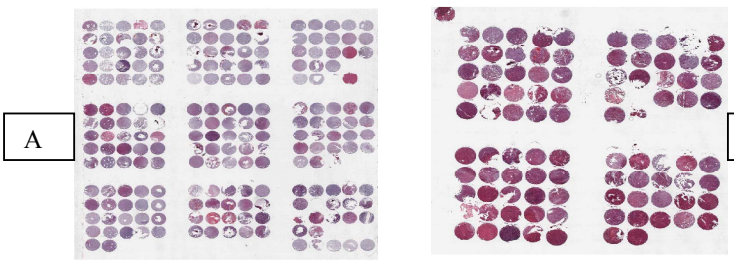


Fig. 1: H&E stained TMA cases of (A) Glioma (B) Meningiome-Schwannoma

INFECTIOUS DISEASES

I. CHLAMYDIASIS

1. Role Of Chlamydial Heat Shock Proteins In Pathogenesis Of Genital Tract Infection In Women

Chlamydial heat shock proteins (cHSP60 and cHSP10) have been implicated in the development of female infertility, since circulating antibodies to these proteins are more common in infertile women than in fertile controls. Overall previous studies suggest that cHSPs specific immune responses play an important role in immuno-pathogenesis associated with chlamydial infection. However, there is no study available wherein the relative expression of cHSP60 and cHSP10 genes at the actual site of infection i.e., cervical epithelial cells has been assessed.

To investigate whether mRNA expression of cHSP60 and cHSP10 genes might be different in fertile and infertile women, their relative transcript levels were assessed using quantitative real-time RT-PCR analysis. After normalization to the level of chlamydial 16S rRNA, a higher transcript level of both cHSP60 ($p=0.007$) and cHSP10 ($p=0.0006$) was found in infertile group than in fertile group (Fig. 1). Further to know whether intracellular cHSP60 and cHSP10 were also overexpressed in infertile women, their expression level was examined by flow cytometry analysis. In contrast to mRNA levels, cervical epithelial cells collected from *Chlamydia* infected fertile group showed significantly higher expression of both cHSP60 ($p=0.0006$) and cHSP10 ($p=0.0041$) in comparison to infertile group . However, when only cHSP positive cells were considered, the mean percentage of cells co-expressing both cHSP60 and cHSP10 was much higher ($p=0.0006$) in infertile group than in fertile group of infected women (Fig. 2). This study shows that in cervical epithelial cells, cHSP60 and cHSP10 had a different pattern of expression in infertile women compared to fertile women reflecting probable difference in the metabolic state of *Chlamydia* with the presence of an abnormal cryptic form of *C. trachomatis* in infertile women. These results strongly support cHSP60 and cHSP10 involvement in immunopathological condition associated with infertility.

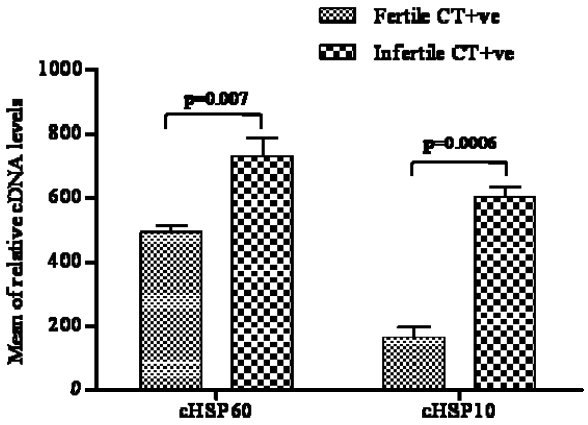


Fig. 1: Real time RT-PCR assay targeting relative transcript levels from the cHSP60 and cHSP10 genes in RNA/cDNA prepared from *C. trachomatis* infected cervical epithelial cells from each of the seven fertile and seven infertile women. Data shown were normalized to the level of *C. trachomatis* 16S rRNA in each sample and mean value in each group was calculated. Assays were repeated three times, with each sample run in triplicate, and all repeats gave essentially identical results. Bars represent standard error.

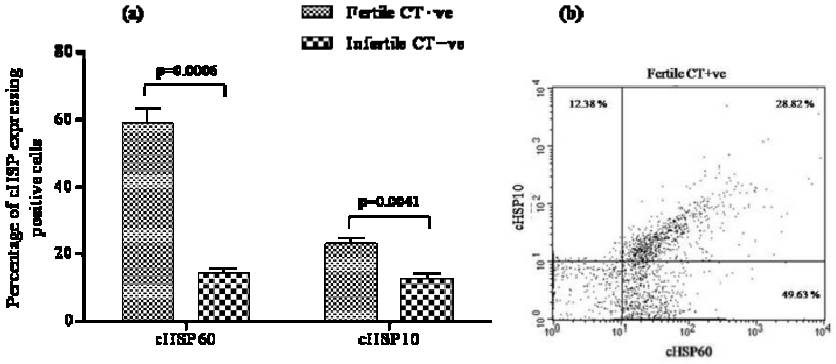


Fig. 2: Flow cytometric assay targeting intracellular levels of cHSP60 and cHSP10 in *C. trachomatis* infected cervical epithelial cells from each of the seven fertile and seven infertile women. Cells were simultaneously stained with monoclonal antibodies of cHSP60 and cHSP10 conjugated with FITC and PE respectively. Data shown were calculated as mean of percent cell population in each group. Appropriate isotype-matched control antibodies were used to rule out non-specific fluorescence. (a) Percentage of cells stained with either cHSP60 or cHSP10. Bars represent standard error. (b) Representative figure (dot plot) showing intracellular levels of cHSP60 and cHSP10 in infected cervical epithelial cells of fertile women.

2. Effect Of Sex Hormones On Induction Of Immunity By Dendritic Cells In Female Reproductive Tract During *Chlamydia trachomatis* Infection

Dendritic cells (DCs) at the mucosal surfaces are central to the generation of immune protection against pathogens. Understanding the mechanism of DC antigen presentation and the role of sex hormones in modulating the immune responses during chlamydial infection will help in understanding of immunopathogenesis of *Chlamydia trachomatis*.

Cervical lavage were collected from symptomatic female patients (age range: 20-40 years) attending the Gynecology Outpatient Department of Safdarjung hospital, New Delhi, India. The female patients were characterized into four groups on the basis of their clinical history; Group I (n = 28) comprised uninfected healthy controls selected from among women attending the family planning clinic; group II (n = 23) comprised asymptomatic *Chlamydia*-positive women without MPC (number of PMNLs <5); group III (n = 25) comprised *Chlamydia*-positive women with MPC (number of PMNLs >30) and *Chlamydia*-positive women with fertility related disorders (FD) (n = 20). *Chlamydia trachomatis* infection mobilized both mDCs and pDCs to the cervical mucosa. Healthy controls had a significantly lower number of mDCs/cervical sample than patients in the *Chlamydia*-positive groups ($p < 0.01$). All the women with MPC and FD had pDC in their cervical samples. Healthy controls had a significantly ($p < 0.001$) lower number of pDCs in their cervical samples than *Chlamydia* infected women. pDCs were recruited more often in women with MPC and FD ($p < 0.05$) and they correlated significantly with the chlamydial load, C-reactive protein levels and cervical interleukin-8 (IL-8) levels (Table 1).

Myeloid dendritic cells

Control	CT + asymptomatic	CT+ with MPC	CT+ with FD
36(0-1256)	1788(0-40170)[a]	720(0-10444)[a,b]	1856(21-8473)

Plasmacytoid dendritic cells

Control	CT + asymptomatic	CT+ with MPC	CT+ with FD
13 (0-1200)	1750 (0-33840)[a]	3890 (1162-80752)[a]	3783 (54-10845)

Note. MPC ; Mucopurulent Cervicitis, FD ; Fertility Disorders

Table 1 : Absolute median number of myeloid and plasmacytoid dendritic cells in cervical lavage samples

All *Chlamydia*-positive women were advised to undergo full antibiotic therapy, and after 4–6 weeks, eight women from each *Chlamydia*-positive group who returned for follow-up were again enrolled. Comparison of paired measurements of mDCs and pDCs in cervical samples in only the patients with chlamydial infection who returned for follow-up evaluation revealed a significant increase in the number of mDCs after therapy, in both *Chlamydia*-infected groups . The median number of pDCs was lower in both the *Chlamydia*-infected groups after therapy.

Further for studying the correlation of chlamydial infectious load with immune factors such as dendritic cells, the *Chlamydia*-positive women were divided into three groups: (a) *Chlamydia*-positive fertile asymptomatic women attending a family planning clinic (n=127), (b) *Chlamydia*-positive fertile women with MPC (thick discharge and inflammation with number of PMNs430) (n=86) and (c) *Chlamydia*-positive women with FD (n=108). *Chlamydia*-positive fertile women showed significantly higher inclusion counts compared with women with FD, showing lower recovery of *Chlamydia* from the cervix of these women . Further, chlamydial IFUs correlated positively with CD8, pDC, IL-8, CRP and IFN-γ in women with MPC. In women with FD, Chlamydial IFUs correlated positively with pDC, IL-10 and estradiol and negatively with CD4 and IFN-γ levels. This data suggests that clinical condition presented is decided by interplay of infectious load and host immune responses.

3. Modulatory Role Of Antichlamydial Agents In *Chlamydia trachomatis* Infection And Their Therapeutic Potential

During chlamydial infection in animals and humans as well as in cell culture systems, wide array of inflammatory cytokines have been implicated to contribute to *Chlamydia*-induced pathologies. Apart from antibacterial activity, its treatment may require modulatory effect on cytokines to clear the pathology associated with *C. trachomatis*. Therefore we further elucidated if azithromycin also modulates the production of cytokines in process of eradication of infection.

The study population was divided into three groups: Group I (n=30) comprised of uninfected healthy controls with no infertility problem; Group II (n=20) comprised of *Chlamydia*-positive women with no infertility problem; Group III (n=18) comprised of

Chlamydia-positive women with infertility. After administration of azithromycin, significant decrease in levels of Interleukin (IL)-8 (P =0.0001 and 0.0003), Interferon-gamma (IFN-γ) (P = 0.01 and 0.01) and Tumor Necrosis factor-alpha (TNF-α) (P =0.01 and 0.01) was observed in cervical secretions of Group II and Group III respectively as compared to levels before therapy in their respective groups (Table 1).

	Group II (n=20)			Group III (n=18)		
	Before treatment	**After treatment**	**P value**	**Before treatment**	**After treatment**	**P value**
IL-1β (pg/ml)	6.5 (UDL-21)	7 (UDL-30)	0.2	14.5 (UDL-38)	11 (UDL-26)	0.3
IL-2 (pg/ml)	5.5 (UDL-17)	5 (UDL-22)	0.8	6 (UDL-32)	5 (UDL-12)	0.5
IL-6 (pg/ml)	3.5 (UDL-27)	4.5 (UDL-23)	0.9	4 (UDL-15)	4 (UDL-25)	0.4
IL-8 (pg/ml)	52 (7-179)	27 (5-88)	0.0001*	69.5 (8-213)	22.5 (7-114)	0.0003*
IL-10 (pg/ml)	7 (UDL-28)	8.5 (UDL-35)	0.3	17 (UDL-40)	18 (UDL-36)	0.4
IL-13 (pg/ml)	4.5 (UDL-16)	5 (UDL-18)	0.8	5.5 (UDL-14)	5.5 (UDL-23)	0.7
IFN-γ (pg/ml)	31 (UDL-97)	12.5 (UDL-64)	0.01*	59 (UDL-178)	21.5 (UDL-134)	0.01*
TNF-α (pg/ml)	18.5 (UDL-79)	8.5 (UDL-32)	0.01*	40 (UDL-116)	14.5 (UDL-56)	0.01*

Table 1: Cytokine concentration in cervical secretions before and after treatment

* Denotes significance level
Cytokine concentration is denoted by median and range in parenthesis
UDL-Under detection limit
Wilcoxon signed rank test was used to compare cytokine concentrations before and after therapy

4. Antichlamydial Drugs: Sensitivity And Emergence Of Resistance In Treatment Failures

C. trachomatis has been historically sensitive to the tetracyclines, macrolides, and fluoroquinolones. Recent reports have suggested increasing *in vitro* resistance. The clinical significance of these findings is unknown. Hence, it would be valuable to understand why recurrent or persistent *C. trachomatis* infection occurs in 10%–15% of women treated for *C. trachomatis* infection. Further study is needed to support or refute the hypothesis that heterotypic resistance of *C. trachomatis* is emerging and is related to increase in clinical treatment failures. The doxycycline drug sensitivity assay was performed on the five isolates using cell culture method for minimum inhibitory

concentration (MIC) determination. The isolates of *C. trachomatis* had shown different susceptibility profile. One of the isolates could not reach the MIC 90 hence, it could be suspected for a heterotypic resistant isolate (Table 1).

S.No.	Isolate No.	Type of isolate	MIC_{50} (µg/ml)	MIC_{90} (µg/ml)	Complete inhibition (At 8µg/ml)
1	CT231	Treatment failure	0.92	7.48	+
2	**CT232**	**Recurrent infection**	**4.0**	**>8**	**-**
3	CT233	Cervicitis	1.3	>8	-
4	**CT235**	**Infertility**	**4.3**	**7.72**	+
5	CT247	Treatment failure	>8	-	-

Table 1: Clinical *C. trachomatis* isolates showing different MIC values for drug doxycycline.

5. Role Of *Chlamydia pneumoniae* In Coronary Artery Disease (CAD) Patients

Atherosclerosis is an inflammatory disease which may be the outcome of responses to microbial antigens. The role of chronic low-grade infection of the arterial wall with *Chlamydia pneumoniae* (*C. pneumoniae*) in the pathogenesis of atherosclerosis has been suggested in a series of epidemiological and pathological studies and they may induce innate immunity, molecular mimicry, and autoimmunity as well as direct infection of tissues. Therefore, the aim of this study was to examine the type of infection in CAD patients in India on the basis of seropositivity (IgG, IgA and IgM) for *C. pneumoniae*, *H. pylori* and CMV, asses the 'p' value and odds of CAD with increasing burden of infection and their association with the level of hs-CRP.

Detection of antibodies for *C. pneumoniae* specific IgG, IgA and IgM, *H. pylori* specific IgG, IgA, and IgM, CMV specific IgG, IgM and hs-CRP, ELISA was performed using respective ELISA kits as per manufacturer's instructions. Level of the hsCRP (more than 3mg/l) in serum was considered as hs-CRP positive in dichotomized result.

The % seropositivity detected in CAD patients for *C. pneumoniae* specific IgG, IgA were significantly more compared to healthy controls (37 v/s 25, 80.2 v/s 39.6), (P = 0.015 and <0.001) respectively. However, for *C. pneumoniae* specific IgM, the % positivity was (25.5 v/s 21.8, P=0.472). Similarly for *H. pylori* specific IgG, IgA and IgM, % positivity for CAD patient's v/s controls were (40.1 v/s 22.4, 51 v/s 29.7, 18.2 v/s 15.1) (P <0.001, <0.001 and 0.582) respectively. CMV specific IgG were also detected significantly high in CAD patients as compared to controls (32.3% v/s 19.8 %; P = 0.010). Presence of hs-CRP was also detected significantly high in CAD patients as compared to controls and % positivity was (59.4 v/s 31.3) (P <0.001) (Table1).

In the combined infection group of *C. pneumoniae* and *H. pylori*, significantly high number 92/192 (47.9%) of CAD patients were found positive for IgA whereas only 32/192 (16.7%) patients had seropositivity for IgG (P <0.001). 37% (71/192) of CAD patients were *C. pneumoniae*-IgA and *H. pylori*-IgG positive while only 46/192 (24%) patients were *C. pneumoniae*-IgG and *H. pylori*-IgA positive (P <0.001).Further *C. pneumoniae* IgA positive CAD patients (Group 2) showed higher levels of hs-CRP (5.18mg/l in serum) than other groups: Group 1 =3.73mg/l, Group 3 =3.36mg/l, Group 4 =4.65mg/l, Group 5 =4.9mg/l and Group 6 =5mg/l (Fig.1).

Infections	Serological and Inflammatory Markers	Patients (n=192)	Controls (n=192)	'P' Value
C. pneumoniae	**IgG**	71 (37)	48 (25)	0.015*
	IgA	154 (80.2)	76 (39.6)	<0.001*
	IgM	49 (25.5)	42 (21.8)	0.472
H. pylori	**IgG**	77 (40.1)	43 (22.4)	<0.001*
	IgA	98 (51)	57 (29.7)	<0.001*
	IgM	34 (18.2)	29 (15.1)	0.582
Cytomegalovirus	**IgG**	62 (32.3)	38 (19.8)	0.01*
	IgM	15 (7.8)	13 (6.8)	0.70
	hs-CRP	114 (59.4)	60 (31.3)	<0.001*

Table 1: Seropositivity for *Chlamydia pneumoniae*, *Helicobacter pylori*, Cytomegalovirus and high sensitive C-reactive Protein in CAD patients and controls.
***Statistically significant**

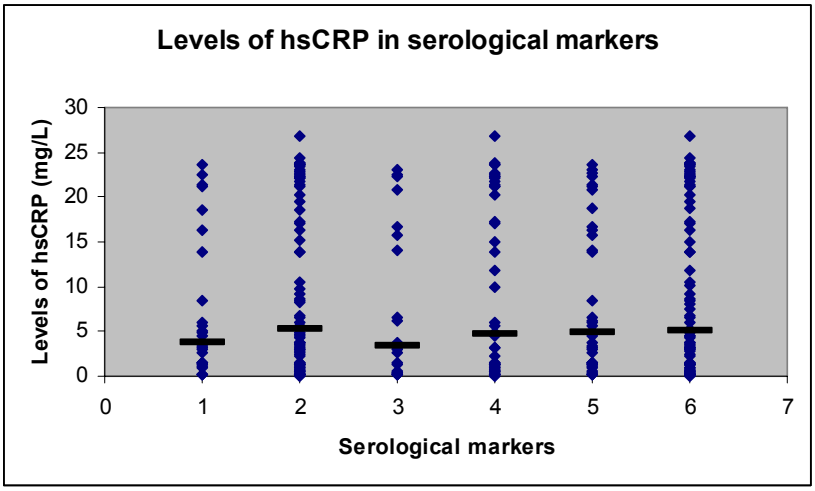


Fig. 1: Levels of high sensitive C - reactive protein according to serologic markers in CAD patients
On Y-axis: Levels of hs-CRP in serological groups.
On X-axis: 1=*C. pneumoniae* IgG CAD patients group, 2= *C. pneumoniae* IgA CAD patients group, 3=*H. pylori* IgG CAD patients group, 4=*H. pylori* IgA CAD patients group, 5=*C. pneumoniae* & *H. pylori* IgG CAD patients group, 6= *C. pneumoniae* & *H. pylori* IgA CAD patients group.

6. Role Of Iron In Pathogenesis Of *Chlamydia trachomatis*

Chlamydia requires iron to complete productive developmental cycle and iron restriction induces persistence of chlamydial infection, simultaneously decreases infectivity as well as virulence, which will be revert back when iron is added. Iron plays a major role in interaction of macrophage with intracellular and extra-cellular pathogens, possibly serving as a catalyst in the generation of toxic oxygen metabolites used in antimicrobial defence and serving as an essential nutrient for intracellular parasite.

Bacterial iron chelator (desferal, deferoxamine mesylate) triggers inflammatory signals, including the production of CXC chemokine IL-8 in human intestinal epithelial cells (IECs) by activating ERK1/2 and p38 kinase pathways. *Chlamydia* enters into persistence stage in the presence of iron-chelating drug (Desferal), thereby showing its dependence on iron for completion of developmental cycle. Persistence can also be induced by antibiosis and tryptophan starvation induced by penicillin G and IFN-γ

respectively. Earlier model of *C. pneumoniae* persistence showed that after IFN-γ and penicillin treatment chlamydia-induced IL-8 expression was inhibited, while it stayed up regulated in iron-depletion. In order to develop appropriate therapeutic regimen, it is essential to define the activation pathways where in iron chelation controls IL-8 induction in chlamydial infection. The mechanism underlying the regulation of iron is still unclear in *Chlamydia* infected cells.

Iron homeostasis of cells is regulated posttranscriptionally by the binding of iron-regulatory proteins (IRP)-1 and -2 with iron-responsive elements (IREs). In iron-starved cells, binding of IRP to IRE stabilizes the transferrin-receptor (TfR) mRNA, major iron transporting protein and inhibits the translation of mRNAs that encode the heavy (H) and light (L) chains of ferritin, iron storage protein, thus promoting cellular iron uptake and preventing iron sequestration .

In this study we showed that expression of transferrin receptor (TfR) was down-regulated; whereas expression of ferritin heavy chain (FHC) was up-regulated in *C. trachomatis* (CT) infected HeLa 229 cells. Expression of TfR in infected cells did not change on addition of iron chelator deferoxamine (DFX) and iron source ferric ammonium citrate. Expression of iron regulatory protein (IRP)-1 predominates over IRP-2 in infected cells. Attenuation in binding activity of IRP- IRE was observed in electrophoresis mobility shift assay of infected cells and is central to iron homeostasis [Fig 1].

Further this study explores that decreased level of intracellular iron in labile iron pool was associated with IL-8 production [Fig 2]. These results suggest that iron homeostasis is modulated in CT infected HeLa 229 cells at the interface of iron acquisition and its commensal utilization.

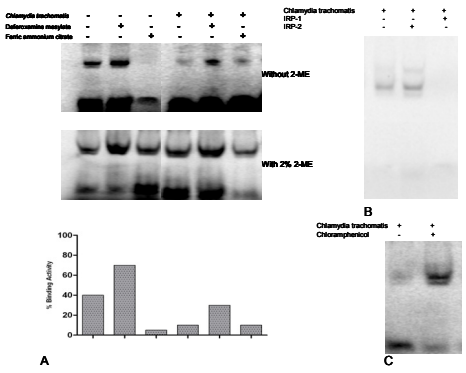


Fig 1

Fig 1: (A) Attenuated binding of IRP-IRE in CT infected cells; (B) Binding completely blocked by IRP-1 antibody; (C) Chloramphenicol restored binding activity in CT infected cells.

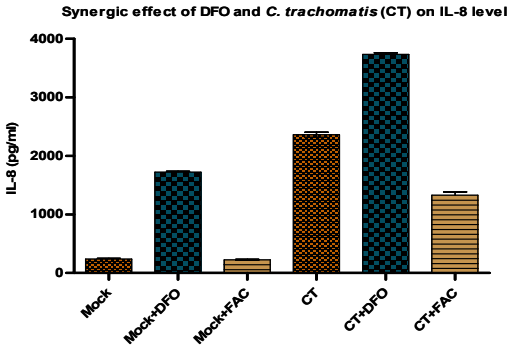


Fig 2

Fig 2: Iron chelator (DFO) acted synergistically with CT to induce IL-8 production in HeLa cells.

7. Inclusion Membrane Proteins And Their Role In Chlamydial Pathogenesis

During developmental cycle, the intracellular bacterial pathogen *C. trachomatis* remains confined within a vacuole known as an 'Inclusion'. With an increase in the number of putative inclusion membrane proteins (Incs) in chlamydial genomes, there is a need for understanding their role in host-pathogen interactions. Thus in this study, we determined the host mucosal and peripheral immune responses to incs (IncB and IncC) of *Chlamydia trachomatis* (CT).

Female patients (n=67) attending the Gynaecology outpatient department of Safdarjung hospital, New Delhi were enrolled for the study and were clinically characterized into two groups; CT-positive fertile women (n=38) and CT-positive infertile women (n=29). Uninfected healthy fertile women attending Family Planning Clinic were enrolled as controls (n=31). In cervical washes and sera, IgA and IgG antibodies to IncB and IncC were detected (Table 1). Using MTT assay, significantly high proliferative responses ($P<0.05$) were observed in inc-stimulated cervical cells and PBMCs from CT-positive fertile women compared to CT-positive infertile women and

controls (Fig. 1). Modulation of cytokines (Interleukin (IL)-1 Beta (1ß), IL-4, IL-5, IL-6, IL-10, Interferon-gamma (IFN-γ), IL-12, Tumor Necrosis Factor-alpha (TNF-α) and Granulocyte macrophage colony-stimulating factor (GM-CSF) in cervical cells and PBMCs on stimulation with IncB and IncC was determined by real-time reverse-transcriptase (RT)-PCR. IFN-γ, IL-12 and GM-CSF were found to be elevated in Inc-stimulated cervical cells and PBMCs of CT-positive fertile women compared to CT-positive infertile women and controls ($P<0.05$). In contrast, IL-1ß, IL-4, IL-5, IL-6 and IL-10 levels were found to be higher in CT-positive infertile women compared to CT-positive fertile women and controls ($P<0.05$). Our data overall suggests that CT incs, IncB and IncC modulate host immune responses and may have a role in protection/ pathogenesis of genital chlamydial infection in women.

Groups	Age	IncB IgG^{+ve}		IncB IgA^{+ve}		IncC IgG^{+ve}		IncC IgA^{+ve}	
	Median (Range)	Serum	Cervical Washes	Serum	Cervical Washes	Serum	Cervical Washes	Serum	Cervical Washes
		n (%)							
Group I (n=31)	24 (21-28)	2(6)	1(3)	1(3)	1(3)	2(6)	1(3)	1(3)	1(3)
Group II (n=38)	26 (22-29)	36(95)[a]	24(63)[b]	23(61)[c]	29(77)[d]	34(89)[e]	27(71)[f]	26(68)[g]	32(84)[h]
Group III (n=29)	27 (22-31)	27(93)[i]	11(38)[j]	17(59)[k]	15(52)[l]	26(90)[m]	13(45)[n]	28(96)[o]	17(59)[p]

Table 1: Prevalence of IncB and IncC specific antibodies in study population

Note values in parenthesis represent corresponding percentages unless otherwise stated.
[a] P=NS as compared to GIII; [b] P=0.0428 as compared to GIII; [c] P=NS as compared to GIII; [d] P=0.0378 as compared to GIII; [e] P=NS as compared to GIII; [f] P=0.0320 as compared to GIII, [g] P=NS as compared to GIII; [h] P=0.0378 as compared to GIII;
[i, j, k, l, m, n, o, p] P<0.0001 as compared to corresponding GI ,
All categorical variables were compared using the $\chi 2$ test.

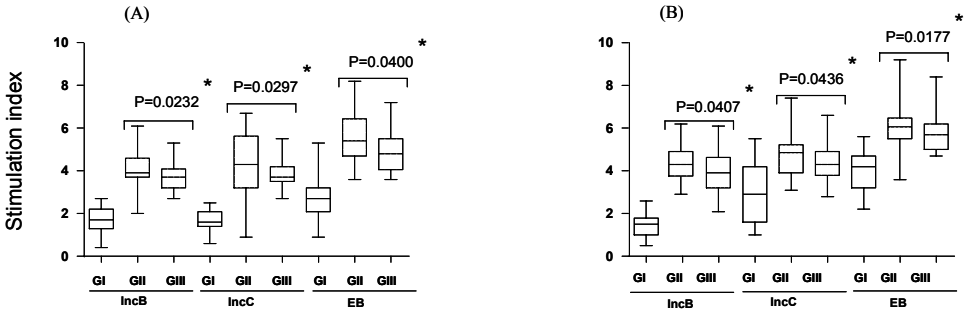


Fig. 1: MTT assay showing proliferative responses
Proliferative responses (stimulation indices) of (A) cervical cells and (B) PBMCs isolated from GI, GII, GIII on stimulation with IncB, IncC and CT EB were estimated by MTT assay.
(A) *Significant difference in proliferative responses in GII with respect to GIII (P= 0.0232, P=0.0297 and P=0.0400 upon stimulation with IncB, IncC and EB respectively)
(B) *Significant difference in proliferative responses in GII with respect to GIII (P=0.0407, P=0.0436 and P=0.0177 upon stimulation with IncB, IncC and CT EBs respectively) where;
Group I (GI) comprised of healthy fertile women with no CT infection. The horizontal line in the middle of the box is the median value of the responses and the lower (upper) is the 25th (75th) percentile. *Significant; PBMCs- Peripheral blood mononuclear cells; Proliferative responses between groups were evaluated using Mann–Whitney *U* test.

8. Role Of Oxidative Stress In *Chlamydia trachomatis* Infected First Trimester Spontaneous Aborters

In spontaneous abortion, expulsion of the uterine content occurs to terminate pregnancy, usually accompanied by uterine contraction while in the case of the missed abortion, dead products of conception are retained in the uterus without bleeding for several weeks. Therefore, the exact mechanism for spontaneous expulsion is not precisely clarified. Many factors including increased free radical activity have been implicated in the pathogenesis of recurrent abortion. However, presently, the etiology of recurrent spontaneous abortion remains unclear and oxidative stress may play a role. The study was continued with the aim to determine if changes in some biomarkers of oxidative stress, *viz.:* nitric oxide (NO), lipid peroxide, malondialdehyde (MDA), superoxide dismutase (SOD) contribute to spontaneous abortion and thereby to define their role in the mechanisms regulating the first trimester pregnancy in *C. trachomatis* positive patients. During the reporting year, endometrial curettage tissue (ECT) samples obtained by dilatation and evacuation and blood were collected from 11 pregnant women undergoing induced abortion (control), 12 patients of spontaneous abortion without vaginal bleeding

and 12 patients of spontaneous abortion with vaginal bleeding attending Safdarjung hospital, New Delhi. All women were in 06-12 weeks of gestation. A part of ECT obtained by curettage was histologically examined for detecting the presence of fetal membranes/ tissues. *In situ* localization of *C. trachomatis* was done using monoclonal anti-human *C. trachomatis* antibody while PCR assay was done for the diagnosis of 364 bp *C. trachomatis*. The levels of nitric oxide, lipid peroxide, malondialdehyde, superoxide dismutase activity were assayed in the sera by using commercial kits. In the spontaneous aborters with vaginal bleeding, SOD activity was significantly lower and concentrations of lipid peroxide and MDA were significantly higher than those in the induced abortion and the spontaneous aborters without vaginal bleeding. Women with missed abortion (spontaneous abortion without vaginal bleeding) showed decreased serum NO levels in comparison to spontaneous aborters with vaginal bleeding. It is speculated that further studies on the role of antioxidant enzyme activities and extracellular matrix of the decidua will be of importance in elucidating the pathogenesis of this disorder.

9. Immunomolecular Expression Of Cyclooxygenases And Prostaglandin Receptors In Endometrial Curettage Tissue Of *Chlamydia Trachomatis* Infected Women During Failed Pregnancy

Maternal infection with *C. trachomatis* is implied as a cause of failed pregnancy in the human female but the pathophysiology is unclear. The microbe or bacterial endotoxin and LPS of gram-negative bacteria may produce phospholipase A2 which is responsible for the release of free arachidonic acid from the membranes. The bacteria or their products may directly or indirectly lead to conversion of arachidonic acid to prostaglandin (PG) E2 and prostaglandin F2α which may induce uterine contraction causing bleeding and miscarriage. The inhibition of prostanoid production with cyclooxygenase (cox) inhibitors attenuates many of the clinical manifestations of bacterial infection. Investigators have failed to draw a correlation between the induction of cox-1 mRNA and the induction of prostanoid production in response to mitogens. Since prostanoids mediate the signs and symptoms of septic shock, stimulate

myometrium and are capable abortifacients, they are likely to be important mediators of *C. trachomatis* induced pregnancy loss. However, the molecular changes underlying the complex transition from uterine quiescence to labour are not fully understood. Our hypothesis is that the differential expression of the PG receptors may be important for regulating uterine activity throughout pregnancy and during spontaneous abortion. In humans, studies have so far been limited to lower segment myometrium biopsies. Thus, till date, there is lack of information regarding expression of EP receptor subtypes in human pregnancy and preterm labour. Therefore, the aim of this study is to study the expression/distribution of cyclooxygenases (cox) 1 and 2 alongwith prostaglandin receptors in *C. trachomatis* infected women undergoing miscarriage. During the period under report, this project was initiated by enrolling 08 women (first trimester) undergoing dilatation and evacuation at Safdarjung hospital, New Delhi. Chlamydial antigen was localized by the immunoperoxidase technique in the endometrial curettage tissue (ECT). *In situ* localization of cyclooxygenases, *viz.:* cox-1 and cox-2 was done in formalin-fixed paraffin ECT sections from *C. trachomatis* infected women. Intense staining for cox-2 was seen in the decidual cells of ECT of *C. trachomatis* positive spontaneous aborters. However, in terms of localization of cox-1, weak immunostaining was observed in the ECT of *C. trachomatis* infected women. The mRNA expression of cox-1 and cox-2 were further standardized in the ECT by RT-PCR to study cyclooxygenase expression at the RNA level.

II. LEISHMANIA

1. Identification Of A Novel Ubiquitin-Like System In The Protozoan Parasite *Leishmania Donovani*

Stage specific transcriptome profiling using *L. donovani* genomic microarray led to the identification of amastigote stage expression of a clone homologous to human Uba5 (Ubiquitin activating enzyme E1) conserved in metazoa and plants. The expression of LdUba5 was validated by real time PCR in both axenic cultures as well as hamster-derived amastigotes. The LdUba5 gene transcripts also were detected in infected bone marrow samples from leishmaniasis patients suggesting a role in the disease pathogenesis in the human host. The LdUba5 was expressed as a histidine tagged protein in bacteria and the recombinant protein was injected into the rabbit for raising polyclonal antibodies against LdUba5. Further, over expression of the LdUba5 was done in *L. donovani* in order to understand its role in *Leishmania* growth. Further studies should illuminate the targets of such modifications that may manipulate the parasite growth/virulence in the mammalian host.

2. Transcriptome Profiling For Identification Of Antimony Resistance Determinants In *Leishmania Donovani* Isolated From Indian Patients Of Kala-Azar

Resistance to antimonials has emerged as a major hurdle to the treatment and control of Visceral Leishmaniasis (VL) or Kala-azar (KA) due to *Leishmania donovani* in India, where over 60% of KA-patients are unresponsive to SbV-treatment. Determinants of resistance in laboratory isolates are partly known, however the mechanism operating in field isolates is not well understood. We present here a comparative transcriptome analysis of antimony resistant and sensitive strains of *L. donovani*.

***Expression profiling in resistant vs sensitive parasite using Genomic Microarray*:**

We developed a microarray for *L. donovani* comprising of 8448 PCR amplified inserts (1.0 to 1.5 kb) from genomic library clones, 24 positive and 12 negative controls, each printed in duplicate. We identified many genes that were uniquely expressed or showed

altered expression in drug resistant strains, indicating their potential role in drug resistance. Genes coding for Protein Surface Antigen 2 (PSA2), Histone (H1), Histone 2A (H2A), Histone 4 (H4), MAP-kinase, and two hypothetical proteins were transcribed more abundantly in the antimony resistant parasite in comparison to sensitive, while genes encoding amino acid transporter, conserved hypothetical protein with ATPase F1/V1/A1 complex signature and conserved hypothetical protein with Myb DNA binding signature showed consistent over-expression in sensitive parasites. Differential expression of all of these genes was validated by semi-quantitative reverse transcriptase (RT)-PCR assay.

Generation of parasites over-expressing PSA / Histones H2A / H4

Genes PSA2/H2A/H4 upregulated in SAG resistant parasite were cloned in pKS Neo vector for overexpression in *L. donovani* promastigotes (1 standard isolate *Ld*S and a SAG sensitive field isolate). PKS-PSA-2++ /PKS-H2A++/PKS-H4++ genes was transfected in *L donovani* and the mutant cell lines were selected under G418 pressure and gene overexpression was confirmed by western blotting using Anti-HA tag antibody.

Functional analysis of parasite mutants overexpressing PSA, H2A or H4

Growth pattern of *Ld* PSA++ / *Ld*H2A++ / *Ld*H4++ was similar to control. However, *Ld* PSA++ and *Ld* H2A++ showed high ED_{50} compared to control while *Ld* H4++ did not.Hence the overexpression of PSA/H2A in *L.donovani* altered the sensitive phenotype to resistant.

Expression analysis of selected genes involved in SAG resistance in field isolates

Based on the current knowledge of Sb (V) metabolism and of the *in vitro* trivalent antimonial [Sb (III)] models of resistance to *Leishmania* spp., we have selected nine genes known to be involved in antimonial resistance and studies are underway for a comparative transcriptomic study on SAG resistant and sensitive *L.donovani* isolates by quantitative Real Time PCR.

3. *In Vitro* Susceptibility Of KA Isolates Towards Antileishmanial Drugs (Miltefosine, Amphotericin B, Paromomycin And Sitamaquine) And Comparison With The Susceptibility SAG

Our studies on *in vitro* drug sensitivities for the current alternative therapies viz. Miltefosine, Amphotericin B revealed that field isolates from HR zone have decreased susceptibility towards these drugs as in case of SAG. *In vitro* susceptibility of isolates to all the three drugs significantly correlated with one another raising the possibility of cross-resistance (Published: Antimicrob Agents Chemotherap, 2009.

Further, We evaluated the intrinsic susceptibility of KA clinical isolates towards paromomycin and sitamaquine. The field isolates (n=20), showed variable susceptibility to these drugs with ED_{50} ± SEM at amastigote stage ranging from 1.07±0.34 to 5.87±0.29 μM for paromomycin and 0.57±0.07 to 3.74±0.78 μM for sitamaquine. Amastigotes were more sensitive to both these drugs in comparison to promastigotes. The data indicates that paromomycin may be a more effective treatment option as parasites from HR and LR region had similar susceptibility to it. In order to investigate whether the drug resistant isolates have the inherent property of producing low levels of NO resulting in decreased drug susceptibility, studies are underway to determine the effect of paromomycin and sitamaquine on the release of NO from infected cells and macrophage activation.

4. Evaluation Of Host-Immuno-Determinants Involved In Pathogenesis Of Kala-Azar And Post-Kala-Azar Dermal Leishmaniasis Using cDNA Array

The interaction between the parasite and the host cell involves complex, multifaceted processes. Majority of the information on related topics with respect to *Leishmania* is based on experimental models; and only limited studies have been carried out with the human host and even those focused on small subsets of genes as suspected immuno-determinants. In this investigation, we take advantage of Nylon array technology for capturing the comprehensive picture of immune parameters in the lesion tissue of Post kala azar dermal leishmaniasis (PKDL) patients, which represents the only reservoir of parasite in India. The snap of global scenario provided opportunities to understand the

immune responses undergoing in chronic lesion tissue. cDNA array data implicates the presence of effector (IFN-γ, TNF-α) and regulatory (IL-10, TGF-ß) molecules together with apoptosis (FasL /TRAIL) and chemokines related genes (MIP-1α, MIP-1α and MCP-1). The data implies that Th1/Th2 paradigm of resistance/susceptibility in human against intracellular parasite *Leishmania* is an oversimplification of complex network of effector/regulatory interactions. Selected genes were validated by Real time PCR/ RT-PCR (IFNγ, TNF-α, IL-10, TGF-ß, CD-40 and MCP-1) and their expression was analysed in lesion tissue compared to control. Several genes with putative role in signaling pathways were also identified as modulated. Role of these genes will further be elucidated by PCR based signaling arrays.

5. Evidence For Involvement Of TNFR1 And TIMPs In Pathogenesis Of Post-Kala-Azar Dermal Leishmaniasis

Semi-quantitative RT-PCR was exploited to analyse the intralesional cytokine gene expression in 14 post-kala-azar dermal leishmaniasis (PKDL) and 10 kala-azar (KA) patients. The data provided evidence for both inflammatory and non-inflammatory responses, as reflected by elevated tumour necrosis factor (TNF)-alpha and interleukin (IL)-10 in PKDL lesions compared with normal skin tissue (n = 6). The ratio of TNF-α: IL-10 message was 2.66 in PKDL cases, substantially higher than in KA (1.18). Investigation of TNF-alpha receptors (TNFR1 and TNFR2) revealed a significant down-regulation of TNFR1 transcript in both PKDL and KA compared with control. In the presence of elevated levels of TNF-alpha transcript, interference with Type 1 effector activity in PKDL may be due to minimal expression of the TNFR1 gene. Investigation of matrix metalloproteinases, known to be induced by TNF-alpha, and the tissue inhibitors of matrix metalloproteinases (TIMPs), provided evidence for the roles of TIMP-1 and TIMP-3 in the pathogenesis of PKDL. (Published: Clin Exp Immunol. 2008).

6. Analysis Of Immune-Determinants In Patients Of Cutaneous Leishmaniasis Caused By *L. Tropica*

Cutaneous leishmaniasis (CL) is caused by different species of *Leishmania*. We have recently established by molecular and immunological tools that *Leishmania tropica*

is the causative agent of cutaneous Leishmaniasis in the endemic region in North-Western part of India. Studies to characterize the immune status of patients in CL caused by *L. tropica* are lacking. The association between localized and circulating levels of immune-determinants in patients of CL was evaluated in the present study. RT-PCR analysis revealed significant up-regulation of interferon (IFN)-γ, interleukin (IL)-1β, IL-8, tumor necrosis factor (TNF)-α, IL-10, and IL-4, in dermal lesions of CL patients at pre-treatment stage (n=31) compared to healthy controls (n=6) (P<0.001). Increased expression of IL-4 was noticed in early lesions including erythematous plaques, ulcerated plaques and granulomatous plaques. At post-treatment stage (n=14), all the 6 cytokines examined were down regulated significantly (P<0.05) in comparison with the pretreatment stage. Increased transcripts of monocyte chemo-attractant protein (MCP)-1 (P<0.001) and inducible nitric oxide synthase (P<0.05) were evident before treatment in tissue lesions and remained high after treatment. At post-treatment stage, serum IL-8 levels declined; however, MCP-1 and NO remained unaltered. IL-8 emerged as an effector immune-determinant in the pathogenesis of CL which may facilitate influx of polymorphonuclear cells at inflammatory site serving as parasite "shelter", while MCP-1 stimulates the parasite killing by macrophages via generation of nitric oxide.

7. Studies On Multilocus Microsatellite Typing (Mlmt) Reveals Genetic Homogeneity Of *Leishmania Donovani* Strains In The Indian Subcontinent

In this collaborative study of population genetics of *L.donovani* parasites in the Indian subcontinent, 132 isolates obtained from patients in Bangladesh, India, Nepal and Sri Lanka suffering from Kala-azar (n=100), post-Kala-azar dermal leishmaniasis (PKDL) (n=25) and cutaneous leishmaniasis (CL) (n=2), and from 5 patients whose clinical patterns were not defined, were analysed by using 15 hyper-variable microsatellite loci. Multilocus microsatellite typing (MLMT) data were analysed by using a Bayesian model-based clustering algorithm and constructing phylogenic tree based on genetic distances. In total, 125 strains from Bangladesh, Bihar (India) and Nepal formed a very homogeneous population regardless of geographical origin, clinical manifestation, and whether they presented *in vitro* or *in vivo* susceptibility to antimonial drugs. Identical

multilocus microsatellite profiles were found for 108 strains, other strains differed in only one marker. Considerably different microsatellite profiles were identified for three Indian strains most closely related to *AQ aqqc* from Kenya, and for four strains from Indian and Sri Lankan CL cases. The circulation of a single homogeneous population of *L. donovani* in Bihar (India), Bangladesh and Nepal is, most probably, related to the epidemic spread of visceral leishmaniasis in this area.

ADULT STEM CELL BIOLOGY

1. Optimal Attenuation Conditions For 3T3 Fibroblasts For Use As Feeder Cells

The primary epithelial stem cells from adult as well as embryonic sources are effectively established in cultures using feeder cells that were growth arrested by either gamma irradiation or exposure to mitomycin C. However, an abrupt over growth of feeders is encountered as a consequence of failed attenuation, particularly while using mitomycin C due to reasons not understood. As a result, there has been a growing controversy on the usefulness of Mitomycin C as an attenuating agent since mytomycin C approach is convenient and does not require high establishment and operational costs as with gamma-irradiation. Earlier it was shown that mitomycin C was effective as an attenuating agent on 3T3 fibroblasts in a 'numerical' dose-dependant manner. Subsequently, experiments were carried out to verify if such differentially growth arrested feeders proportionately stimulate the proliferation of human epidermal keratinocyte stem cells.

This study is planned to evaluate the effectiveness of various numerical doses of mitomycin C in the medium on 3T3 fibroblast in stimulating human epidermal keratinocyte stem cells.

There was a highly significant negative correlation ($P<0.001$) between the cell numbers of 3T3 and keratinocytes while including all cell count-data on days 6 or 9 (Fig. 1) indicating that the superior growth supporting activity of 3T3 feeders could be achievable with concomitant sequential increase in the extent of attenuation through differential numerical dosing. However, the negative correlation was insignificant when calculated independently for days 3 or 9, while it was significant ($P<0.05$) for day 6 showing that maximal growth was obtainable by 6 days of co-culture. The calculated R^2 values for 3T3 and keratinocyte stem cells were 0.94 and 0.8.

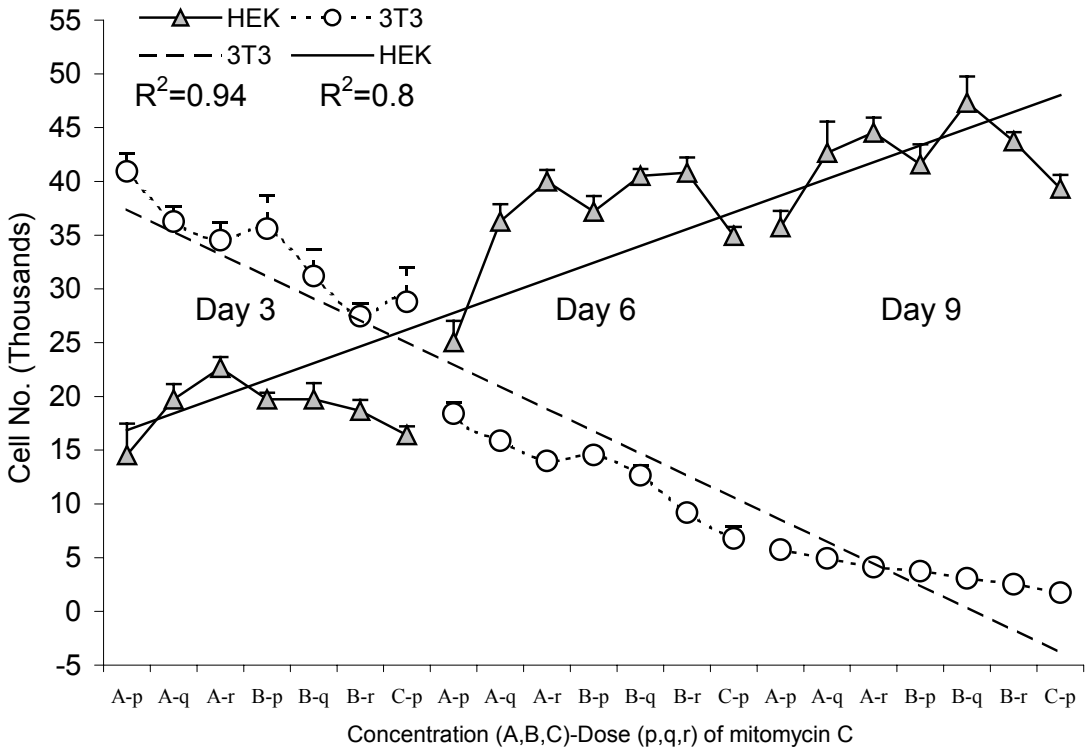


Fig. 1: Line diagram plotted with number of keratinocyte stem cells and 3T3 cell number obtained with 3T3 cells attenuated with concentration-dose combinations of A and B μg Mitomycin C at doses of p, q and r μg and C μg Mitomycin C at a dose of p μg for a 2-hours pulse as represented by A-p, A-q, A-r, B-p, B-q, B-r and C-p Mitomycin C on x-axis and cell numbers representing 3T3 cells (3T3, broken lines) and keratinocytes (HEK, solid lines) on days 3, 6 and 9 (D3, D6, D9), respectively, on y-axis, showing day-wise change in cell number. The lines were interrupted to distinguish observations on each time point. Linear trend lines representing 3T3 cell number (3T3, broken bold line) and keratinocytes (HEK, solid bold line), respectively, were plotted by calculating the least squares fit through cell number data points using regression analysis. Each point is the mean of cell counts from 3 wells and standard deviation is shown as error bars on v-axis.

The results indicated that successful attenuation is dependent on numerical dosing with concomitant optimization in the stimulation of keratinocyte cell proliferation. Further supportive work on colony forming efficiency and mitotic index estimation using BrdU labeling on keratinocytes grown over differentially attenuated feeders is underway.

2. Investigation Into The Utility Of A Patented Synthetic Thermo-Reversible Hydrogel Polymer As Supportive Matrix Towards The Development Of 3-D Composite Skin For Application In Wound Healing And Other Dermatological Disorders

Tissue engineering involving in vitro construction of 3-dimensional cellular structures using various matrix materials is one of the recent approaches for wound healing. Such constructs utilize certain biological matrices that are either animal sourced or semi-synthetic and associated with several safety issues. A purely synthetic non-toxic thermoreversible gelation polymer hydrogel (TGP), Mebiol gel, developed by Prof.Yuichi Mori, at the Waseda University, Tokyo, Japan, has been proven to support hepatic progenietor cells, corneal limbal cells and several other cell lines.

This study is undertaken to verify the potential of Mebiol gel to support without the animal sourced feeder cells the growth and differentiation of primary human epidermal keratinocyte stem cells.

Experiments were performed using (TGP) prepared in keratinocytes medium for primary human keratinocyte stem cells. Several permutations of TGP-keratinocyte 3-D structural organizations were attempted out of which TGP overlay on keratinocytes plated on plastic surface showed some indications of cellular movement, while the cells seeded as concentrated pellet in between 2 discs of gels in cell inserts showed marked migrations towards the periphery of the discs (Figs. 1-2). On the other hand, keratinocytes seeded over conventional culture surface without 3T3 serving as control showed cytoplasmic enlargement (differentiated).

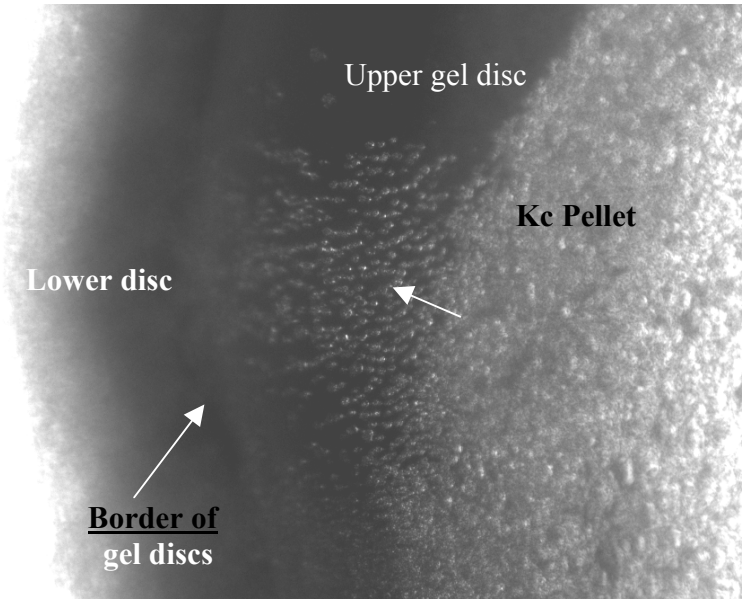


Fig. 1: A stream of cells migrating from the core of keratinocyte pellet (KC Pellet) towards the periphery.

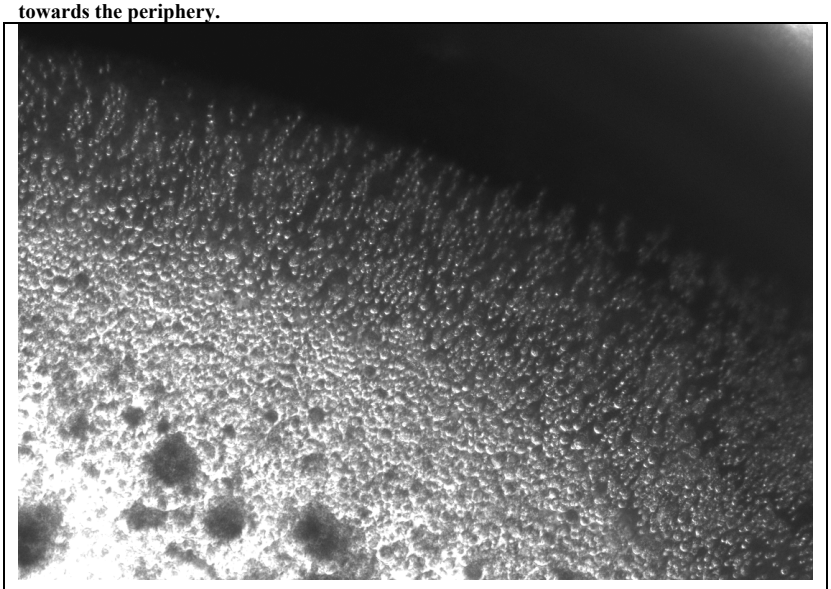


Fig. 2: The small spherical keratinocytes emerging out of a concentrated keratinocyte pellet sandwiched between two discs of Mebiol gel.

The negative results from the previous experiments and the present experiments hint suggest that the TGP perhaps specifically stimulates only those keratinocytes that have inherent stemness. Experiments to show mitotic activity in keratinocytes a sandwich organization. It is felt that the composition of the KCM needs to be modified to stimulate cell proliferation and various modalities are being worked out in this direction.

ENVIRONMENTAL TOXICOLOGY

1. Health Hazards Of Phthalates Vis-À-Vis Idiopathic Male Infertility

Several studies have shown the adverse effects of butyl benzyl phthalate (BBP) on both size and sperm production in rat testes. However, there have been conflicting reports about effects of phthalates on human reproductive system. Additionally phthalates along with numerous other chemicals such as organochlorine pesticides, polychlorinated biphenyls (PCBs), bisphenol-A etc., are reported to exhibit estrogenic activity. These may bind with hormone receptor in the body and inhibit the activity of natural hormone or elicit hormone like effects by themselves. This study has been undertaken with a view to study links between exposures to xenoestrogens with the reported declining semen quality in human beings. There may be several mechanisms that may be responsible for reproductive abnormalities induced by these chemicals. This study has been planned to detect, estimate and correlate the levels of different phthalates and their metabolites in urine samples of infertile patients with abnormalities in biochemical, functional, hormonal as well as ultrastructural parameters of sperms.

During the period under report, 50 samples of semen, blood and urine were collected from male patients reporting primary/secondary infertility. Samples of blood and urine were stored at -20ºC. The semen samples were subjected for assessment of different physical, functional, biochemical and morphological parameters as per the WHO protocol. Semen samples were further processed for electron microscopy. On electron microscopy, the samples revealed changes in microtubular pattern, head and neck abnormalities, multiple heads, mitochondrial abnormality, etc. In addition to morphological analysis, attempts were made to estimate levels of testosterone and estradiol in the serum samples. Mean testosterone level was found to be 5.06 units in the patient group in comparison to 7.44 units in the control samples indicating a significant decline in infertile group. The decline showed positive correlation with increasing potential of occupational phthalate exposure. The testosterone levels also showed corresponding decline with sperm count and motility morphology. The estradiol levels were consistently higher in patient group (avg. 28.89 units) in comparison with control

group (avg. 13.43 units). While the data was highly skewed, the median levels provided more rational measure of central tendency. The median levels were similar in low and medium risk category; there was a significant decline in the high risk category. Further standardization of procedures for estimation of phthalates by Gas Chromatography has been initiated. The results on hormonal, morphological and functional studies will be correlated with the levels of phthalates and their metabolites estimated by GC-MS.

2. Dynamics Of Ultra-Structural And Immunological Events In Response To Treatment In Different Forms Of Psoriasis

The morphology of psoriatic skin is characterized by epidermal thickness and parakeratosis, a pronounced dermal vascular plexus, and the presence of inflammatory cells in the superficial dermis and epidermis. Increased polymorphonuclear leukocyte levels damage surrounding tissue by releasing reactive oxygen species produced via NADPH oxidase / myeloperoxidase and proteolytic enzymes. Increased production of oxygen metabolites is a common feature of most human diseases including psoriasis and it usually triggers an upregulation of the antioxidant capacity, which is overwhelmed. When the oxidative stress develops, it leads to the oxidative damage of lipids and proteins. Alterations in plasma lipid and lipoprotein composition including a tendency toward an increase in total cholesterol and triglyceride and decrease in high-density lipoprotein cholesterol levels suggest that psoriasis may associate with the disorders of lipid metabolism. The exact aetiology of psoriasis is unknown, however, genetic, immunologic and metabolic mechanisms including abnormalities in essential fatty acid metabolism, free radical generation, lipid peroxidation, and release of lymphokines have been proposed.

This study has been designed to incorporate all these factors including ultrastructural changes. During the year under report, 20 patients were enrolled for the study. They were clinically examined and P.A.S.I. (Psoriasis Area Severity Index) score was calculated. 3 mm skin punch biopsies were taken from the margin of the lesional skin of the patient and uninvolved normal skin of the patient as control. The neutrophils were observed in upper dermis, basal layer of epidermis, as well as in higher layers of

epidermis with occasional loss of basal layer integrity. Basal lamina split was seen more frequently. Higher turnover of epidermal cells resulted in pushing of basal melanocytes to higher layers. Rete pegs were dilated and surrounded by macrophages. Langerhans cell were regularly found which were confirmed by the presence of Birbeck granule. Dividing keratinocytes were observed in the lower layer of the psoriatic epidermis. In addition immunohistochemical procedures were standardised for localization of 8-OHdG, 4-HNE and DiBrY to assess oxidative stress against DNA, proteins and lipid.

3. Assessment Of Pesticide Exposure In Tea Garden Workers Of North-Eastern State Of India (HEBM)

More than 50% of over 1.1 million workers in the labour intensive tea industry are women. The workers involved in activities such as pesticides spray, mixing and storing, rarely use any safeguard, take food without washing of hands and may even use jerry cans and bags (emptied after usage of pesticide) for storage of household food grain. An ICMR funded extramural study cross-sectional study was designed to assess the pesticide exposure of female workers employed in tea plantations during tea-farming activities in north-eastern region of India.

During the year under report, 25 samples of placenta, cord blood and maternal blood were collected from Safdarjang hospital. Similarly 15 respective samples were collected from Assam Medical College, Dibrugarh. The subjects were assessed for occupation, family background, obstetrics, clinical and gynaecologic history, dietary and smoking habits etc., socio-economic background, and possibilities of exposure to pollution, delivery and sample details using a detailed proforma form. The samples of placenta as well as blood were processed for homogenization and extraction of organic pollutants. Simultaneously, a cocktail of commonly used organochlorine and organophosphorus pesticides and fungicides used in agriculture and for domestic purpose was selected for multi-residue analysis. Initially, Deltametherin Fenverate, Flufenacet, Endosulphan, Malathion, Tebuconazole, Dimethoate were used for standardization. After a large number of repeated trials, the analytical conditions for HPLC were optimized and standardized so as to distinctly separate the selected pollutants. The pesticides were

separated by reversed phase HPLC using a Shimadzu high performance liquid chromatography System Model LC-20AD.

Initial investigations have revealed an interesting dichotomy between subjects from Delhi and N.E. region in terms of literacy, food habits, and usage of cooking fuel, mosquito repellents and consumption of tobacco products. Differences were also observed in the health status of subjects between Delhi and N. E. region in terms of haemoglobin, blood pressure, baby weight and ponderal index etc. The pollutants present in the samples were identified by comparing the retention time of the peaks observed in the chromatogram of the sample with those recorded for the pesticide standard analysed with the same HPLC under similar analytical conditions. Analysis of the chromatograms showed presence of or more peaks. Some of these peaks could be recognised based on the RT of the standards. These included Dimethoate, Tebuconazole, Flufenacet, Malathion and Endosulfan. However, several of the peaks could not be recognized at the moment. Attempts would be made to recognize these peaks with fresh and more comprehensive array of pesticide standards.

ACADEMIC ACTIVITIES

AWARDS & HONOURS

1. Dr. Sunita Saxena received **K.C. Basu Mullik Award** for 2008 by Indian Association of Pathologists and Microbiologists at Chennai for Best Research Work "Study of molecular functional pathways associated with esophageal cancer in North East India".

2. Dr. Poonam Salotra elected **Fellow of the National Academy of Sciences, India**, in the year 2008.

3. Paper entitled "**Ultrastructural studies in mice tail model of psoriasis**" by Dr. AK Jain was conferred best oral paper in Biological Science Category at National Conference on Electron Microscopy and XXX Annual Meeting of Electron Microscope Society of India, Bundelkhand University, Jhansi in January 17-20, 2009.

4. Dr. Poonam Salotra invited by **WHO as member of Expert Committee** for Leishmaniasis control, TDR- WHO in 2009.

5. Mr. Paresh Sharma, PhD student and SRF received the **Best Poster Presentation Award at the National Conference on Emerging Trends in Life Sciences Research** held at BITS –Pilani in March 2009.

6. Mr. Rishen Gupta, PhD student and SRF received the **Best Oral Presentation award at the National Conference on Emerging Trends in Life Sciences Research** held at BITS –Pilani in March 2009.

FELLOWSHIPS

1. Dr. AP Singh awarded **Indo-US Research Fellowship** at Advanced Technology Center, Tissue Array Research Lab, National Cancer Institute, NIH, Gaithersburg, USA from Aug1-Oct 31, 2008

SCIENTISTS TRAINED ABROAD / CONFERENCE/ WORKSHOP

1. Dr. Sunita Saxena invited to deliver talk on "Differential gene expression in familial and tobacco associated esophageal cancer in north-east region of India" at BIT's World Cancer Congress held at Shanghai, China during 12^{th} -17^{th} June 2008.

2. Dr. Poonam Salotra was an invited speaker in the scientific meeting: IFoLeish-2008 (Interdisciplinary Forum on Leishmaniasis) held at Heidelberg, Germany in April 2008.

3. Dr. Poonam Salotra visited the Institute of Microbiology, Charite University, Berlin, Germany for work under Indo-German project in June 2008.

4. Dr. Poonam Salotra participated in Workshop for the European Commission project-RAPSODI held at Carros, France in Jan. 2009.

5. Dhiraj Kumar, PhD student and SRF, was awarded Bill and Melinda Gates Global Health Travel Award to attend the Keystone Symposium on "Drug discovery for protozoan parasites" held at Colorado, USA in March 22-26, 2009.

6. Rajesh Kumar, PhD student and SRF attended a course on Molecular Biology of *Leishmania* conducted by ICGEB at Trieste, Italy in Oct. 2008.

EXTRAMURAL PROJECTS

NEW PROJECTS

1. Characterisation of host immune factors associated with progression of superficial TCC of bladder by microarray analysis.
   Dr. Sunita Saxena (2008-2011).

2. New Tools for monitoring drug resistance and treatment response in visceral leishmaniasis in the Indian subcontinent.
   Dr. Poonam Salotra - European Commission (2009-2012).

3. Pre-clinical studies of a PSA based human vaccine candidate targeting visceral , cutaneous and muco- cutaneous leishmaniasis and development of the associated procedures for further clinical trials.
   Dr. Poonam Salotra - European Commission (2009-2012).

4. Parasite surface antigen-2(PSA-2) of *Leishmania donovani*: Studies on its role in parasite virulence, drug resistance and modulation of host macrophage function.
   Dr. Poonam Salotra - Department of Science and Technology (2008-2011).

ONGOING PROJECTS

1. Establishment of breast cancer cell lines from primary breast tumours.
   Dr. Sunita Saxena, Dr. Sujala Kapur, Dr. Usha Agrawal – DBT (2008-2011).

2. Study on gene expression and hypermethylation profiles in early onset breast cancer.
   Dr. Sunita Saxena, Dr. Sujala Kapur, Dr. BSA Raju - ICMR Multicentric Task Force Project (2005-09).

3. Microsatelite instability in androgen receptor gene, p53 gene polymorphisms/mutations and expression profile of mismatch repair genes in prostate cancer.
   Dr. Sunita Saxena - DST (2005-2008).

4. Role of tobacco use in causation of cancer in north-east India.
   Dr. Sunita Saxena, Dr. Sujala Kapur, Dr. Usha Agrawal - ICMR Multicentric Task Force Project (2005-09).

5. Effect of pesticide exposure in causation of cancer in north-east India.
Dr. Sunita Saxena, Dr. Sujala Kapur, Dr. Usha Agrawal - ICMR Multicentric Task Force Project (2005-09).

6. Analysis of host immuno-determinants involved in the pathogenesis of Indian cutaneous leishmaniasis exploiting cDNA microarray.
Dr. Poonam Salotra - ICMR (2007-2010).

7. Evaluation of host immunodeterminants involved in the pathogenesis of kala-azar and post kala-azar dermal leishmaniasis using cDNA array.
Dr. Poonam Salotra - DRDO, Ministry of Defence (2006-2009).

8. Assessment of pesticide exposure in tea garden workers of north-eastern state of India (HEBM)
Dr. AK Jain - ICMR (2008-2010).

9. Investigation into the utility of a patented synthetic thermo-reversible hydrogel polymer as supportive matrix towards the development of 3-D composite skin for application in wound healing and other dermatological disorders.
Dr. LK Yerneni - ICMR (2007-2010).

COMPLETED PROJECTS

1. Comprehensive study of carcinoma oesophagus at northeast India - multidiscipline approach.
Dr. Sunita Saxena, Dr. Sujala Kapur - ICMR Multicentric Task Force Project (2004-08).

2. Molecular characterization of *Leishmania* parasites isolated from dermal lesions of PKDL patients in India.
Dr. Poonam Salora - Indo-German (2005-2008).

3. Discovery of virulence-related genes in *Leishmania donovani* using a genomic microarray.
Dr. Poonam Salora - Indo-US (2004-2008).

PUBLICATIONS

In Journals

1. Tyagi I, Agarwal U, Amitabh V, Jain A K, Saxena S. Thickness of glomerular and tubular basement membranes in preclinical and clinical stages of diabetic nephropathy. ***Indian Jr of Nephrology***, 18(2):60-5, 2008.

2. Sharma Monika, Chintamani, Saxena S, Agrawal Usha. Squamous cell carcinoma arising in unilateral Warthin's tumor of parotid gland. ***J of Oral and Maxillo Facial Pathology,*** 12 (2), 2008.

3. Murthy NS, Chaudhry K, Nadayil D, Agarwal UK, Saxena S. Changing trends in incidence of breast cancer:Indian scenario. ***Ind J of Cancer***, 46(1):73-4, 2009.

4. Bhengraj AR, Dar SA, Talwar GP, Mittal A. Potential of a novel polyherbal formulation BASANT for prevention of *Chlamydia trachomatis* infection. ***International Journal Antimicrobial Agents*****,** 32:84-8, 2008.

5. Jha HC, Mittal A. Coronary artery disease patient's first degree relatives may be at a higher risk for atherosclerosis. ***Int Jr of Cardiology*****,** doi: 10, 1016/j.ijcard 2008. 03 .031. 2008.

6. Agarwal T, Vats V, Wallace P, Singh A ,Salhan S, Mittal A. Recruitment of myeloid and plasmacytoid dendritic cells in cervical mucosa during *C. trachomatis* infection. ***Clin Micobiol Infec*****,** doi: 10.1111/j.1469-0691.2008.02113x, 2008

7. Dutta R, Jha R, Salhan S, Mittal A. *Chlamydia* specific heat shock protein 60 antibodies can serve as prognostic marker for chronic *Chlamydia* infection. ***Infection***, 36: 374-378, 2008.

8. Jha HC, Prasad J, Mittal A**.** High IgA seropositivity for combined *Chlamydia pneumoniae*, *Helicobacter pylori* infection and high sensitive C-reactive protein in coronary artery disease patients in India can serve as atherosclerotic marker. ***Heart and Vessels*****,** 23:390-6, 2008.

9. Srivastava P, Jha R, Salhan S, Mittal A**.** In Infertile women, cells from *Chlamydia trachomatis* infected site release higher levels of interferon- gamma, interleukin-10 and tumor necrosis factor-alpha upon heat shock protein stimulation than fertile women. ***Reproductive Biology and Endocrinology*****,** 6:20, 2008**.**

10. Srivastava P, Gupta R, Jha HC, Bhengraj AR, Jha R, Salhan S, Mittal A. Serovar specific immune responses to peptides of variable regions of *Chlamydia*

trachomatis major outer membrane protein in serovar D infected women**.** ***Clinical and Experimental Medicine,*** 8: 207-15, 2008**.**

11. Jha H, Prasad J, Srivastava P, Sarkar R, Mittal A**.** *Chlamydia pneumoniae* IgA and elevated level of IL-6 may synergize to accelerate coronary artery disease outcome. ***Jour of Cardiology***, 52: 140-45, 2008.

12. Aggarwal T, Vats V, Salhan S, Mittal A. Role of cervical dendritic cell subsets, costimulatory molecules,cytokine secretion profile and Beta-estradiol in development of sequelae to *Chlamydia trachomatis* infected women. ***Reproductive Biology & Endocrinology***, 6:46, 2008**.**

13. Aggarwal T, Gupta R, Dutta R, Srivastava P, Bhengraj R, Salhan S, Mittal A. Protective or pathogenic immune response to genital chlamydial infection in women-A possible role of cytokine secretion profile of cervical mucosal cells. ***Clinical Immunology***, doi:10.1016/j.clin.10.004, 2008.

14. Gupta R, Jha R, Salhan S, Eickhoff M, Krupp G, Mittal A**.** Existence of plasmid-less clinical isolate of *Chlamydia trachomatis* in India is a cause of concern. ***Inter Jour Microbiology*****,** 5(2):1-8, 2008.

15. Srivastava P, Jha HC, Salhan S, Mittal A**.** Azithomycin treatment modulates cytokine production in *Chlamydia trachomatis* infected women**.** ***Basic and Clinical Pharmacology & Toxicology,*** doi:10.1111/j.1742-7843.2009.00395.x. 2009.

16. Aggarwal T, Vats V, Salhan S, Mittal A**.** Determination of chlamydial infectious load and immune parameters in asymptomatic, symptomatic and infertile women. ***FEMS*****,** 55: 250-57, 2009**.**

17. Jha H, Prasad J, Mittal A**.** Why first degree relatives of coronary artery disease patient's have *Chlamydia pneumoniae* infection? ***Int Jr Cardiology*****,** 2009**,** doi:10.1016/j.ijcard.12.062. 2008.

18. Jha H, Prasad J, Mittal, A. Association of plasma circulatory markers, *Chlamydia pneumoniae* and high sensitive C-reactive protein**.** ***Mediators of Inflammation,*** doi:10.1155/561532/2009**.**

19. Ansari NA, Katara GK, Ramesh V, Salotra P. Evidence for involvement of TNFR1 and TIMPs in pathogenesis of post-kala-azar dermal leishmaniasis. ***Clin Exp Immunol,*** 154:391-8, 2008.

20. Ansari NA, Ramesh V, Salotra P. Immune response following miltefosine therapy in a patient with post-kala-azar dermal leishmaniasis. ***Trans R Soc Trop Med Hyg,*** 102:1160-2, 2008.

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22. Ansari NA, Kumar R, Raj A, Salotra P. Elevated levels of IgG3 and IgG4 subclass in paediatric cases of kala-azar. ***Parasite Immunol***, 30: 403-9, 2008.

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27. Ramesh V, Ansari NA, Jain RK, Salotra P. Oral miltefosine in the treatment of post-kala-azar dermal leishmaniasis. ***Clin Exp Dermatol***, 33:103-5, 2008.

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31. Das RP, Jain AK, Ramesh V. Current concepts in pathogenesis of psoriasis*.* ***Ind J Dermatol***, 54: 7-12, 2009.

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34. Kumar Ashok, Yerneni LK. Semi-automated relative quantification of cell culture contamination with *Mycoplasma* by photoshop-based image analysis on immunofluorescence preparations. ***Biologicals,*** 37:55-60, 2009.

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39. Avninder S, Kumar J, Kapur S, Ramesh V. Subcutaneous panniculitis-like T-cell cutaneous lymphoma. ***Indian J Dermatol Venereol Leprol***, 74(2): 151-3, 2008.

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1. Jain AK. Declining semen quality : Role of persistent organic pollutants. In: Sharma RS, Rajanna A and Rajalkashmi M, eds. ***Recent Advances and Challenges in Reproductive Health Research,*** New Delhi, Indian Council of Medical Research, pp. 437 –56, 2008.

2. Shivani Negi, AK Mishra, Anju Bansal, Amar Bhatnagar, Dinesh Bhatnagar, Chintamani, Sunita Saxena : Statistical Considerations in Breast carcinoma-A Study on Association of Androgen Receptors with Clinical Response. Population, ***Poverty and Health: Analytical Approaches***. Chapter 20:277-284.

ACCEPTED FOR PUBLICATION

In Journals

1. Chattopadhyay I, Phukan R, Singh A, Vasudevan M, Purkayastha J, Hewitt S, Kataki A, Mahanta J, Kapur S, Saxena S. Molecular profiling to identify molecular mechanism in esophageal cancer with familial. ***Oncol Reports*****.**

2. Agrawal Usha, Sharma Monika, Bhatnagar Dinesh, Saxena S. Leydig cell tumor - An unusual presentation. ***Ind J of Pathol and Microbiol****.*

3. Singh A, Kapur S, Chattopadhyay I, Purkayastha J, Sharma J, Mishra A, Hewitt SM, Saxena S. Cytokeratin immunoexpression in esophageal squamous cell carcinoma of high-risk population in northeast India. ***Applied Immunohistochemistry & Molecular Morphology.***

4. Kaushal M, Chattopadhyay I, Phukan RK, Purkayastha J, Mahanta J, Kapur S**,** Saxena S. Contribution of germline BRCA2 sequence alterations to risk of familial esophageal cancer in high-risk area of India. ***Diseases of the Esophagus*****.**

5. Gupta R, Salhan S, Mittal A. Seroprevalance of antibodies against *C. trachomatis* inclusion protein B & C in infected women. ***Jr of Infection in Developing Countries*****,** 2009.

6. Jha R, Bas S, Salhan S**,** Mittal A**.** In infertile women, cervical epithelial cells from *Chlamydia trachomatis* infected site co-express higher level of chlamydial heat shock protein 60 and 10 than in fertile women**.** ***Gynaecologic and Obstetric Investigation,*** 2009.

7. Gupta R, Salhan S, Mittal A. Mucosal and peripheral immune responses to chlamydial inclusion membrane proteins B and C in women infected with *Chlamydia trachomatis***.** ***Reproductive Biology& Endocrinology*****,** 2009.

8. Vardhan H, Dutta R, Vats V, Gupta R, Jha H, Jha R, Srivastava P, Bhengraj A, Mittal A. Elevated IL-8 promotes cellular sustenance not the proliferation of *Chlamydia trachomatis* infected HeLa 229 cells. ***Mediators of Inflammation*****,** 2009.

9. Singh Ranvir, Saluja Sumita, Jayaraman S. Evaluation of Human T-cell Lymphotropic virus type-1 (HTLV-1) in blood donors and patients with hematological malignancies from Delhi, India. ***IJMR*****,** 2009.

10. Chakraborty Anurupa, Mishra AK, Soni Abha, Regina Thodum, Bhatnagar D, Bhatnagar A, Chintamani, Saxena S. VDR gene polymorphism(s) and breast cancer risk in north Indian population. ***Cancer Epidemiology***.

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2. Yadav DS, Devi Th. Regina, Mishra AK, Sharma J, Verma Y, Jamoivia E, Kapur S, Saxena S. Genetic polymorphisms of CYP1A1 genotypes in various ethnic groups of India. International Symposium on Ethics Culture and Population Genomics & 34th Annual Conference of the Indian Society of Human Genetics" organized by ISHG and ASI in New Delhi from March 17-20, 2009.

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8. Chattopadhyay I, Singh A, Kapur S, Saxena S. Differential expression of MAPK and GPCR pathway in esophageal cancer of North-east region of India in Human Genome meeting (HGM2008 Workshops), Hyderabad, September 27-30, 2008.

9. Rakhshan Ihsan, Kaushal M, Soni A, Devi TR, Yadav DS, Mishra AK, Sharma J, Behera D, Jaiswal A, Gupta K, Zomawia E, Kapur Sujala, Saxena Sunita. Significance of TP53 codon 72 polymorphism in breast and lung cancer showing different xenobiotic potential spectrum in Human Genome Meeting 2008, Hyderabad.

10. Ihsan Rakhshan, Sharma J, Behera D, Jaiswal A, Gupta K, Zomawia E, Kapur Sujala, Saxena Sunita. Influence of germline polymorphisms of TP53, GSTM1 and GSTT1 genes in lung cancer risk organized by BITS, Pilani in March 6-7, 2009.

11. Chauhan Singh Pradeep, Bhushan Bharat, Verma Saurabh, Saluja Sumita, Sharma Monica, Mishra Ashwini Kumar, Mittal Vishakha, Kabra Meghana, Bhasin Sumita, Gupta Dipendra Kumar, Kapur Sujala. Expression of Ki67 as a prognostic indiacator in acute leukemia in National Conference on Emerging Trends in Life Sciences Research held on March 6th & 7th, 2009 at BITS, Pilani , Rajasthan.

12. Chauhan Pradeep Singh, Bhushan Bharat, Verma Saurabh, Saluja Sumita, Sharma Monica, Mishra Ashwini Kumar, Mittal Vishakha, Kabra Meghana, Kapur Sujala. Immunophenotypic and clinical findings in adult acute myeloid leukemia with FLT3 internal tandem duplication. Proceedings of 13th Human Genome Meeting (HGM 2008), Hyderabad, India, 2008.

13. Bhushan B, Chauhan P S, Saluja S, Mishra A K, Bhasin S, Gupta D K, Siddiqui S, Kapur S. NF-kB signaling pathway in acute leukemia: A study on expression of cell survival and proliferative genes by Real Time RT-PCR. Proceedings of 13th Human Genome Meeting (HGM 2008), Hyderabad, India, 2008.

14. Mishra AK, Pandey CM. Some epidemiological models for the parasitic infections: Comparison between traditional and hierarchical logistic regression methods. Proceedings of the 56th Session of the International Statistical Institute (I.S.I.), Lisbon Session 2007, 22nd -29th August 2007.

15. Shivani Negi, AK Mishra, Anju Bansal, Amar Bhatnagar, Dinesh Bhatnagar, Chintamani, Sunita Saxena : Statistical Considerations in Breast carcinoma-A Study on Association of Androgen Receptors with Clinical Response. Population. Proceedings of the 29th National Conference of the Indian Association for the Study of Population and National Seminar on Recent Statistical Technique for Data Analysis" at Banaras Hindu University (B.H.U.), Varanasi, 26th – 28th October, 2007.

PARTICIPATION IN SCIENTIFIC ACTIVITIES

Dr. Sunita Saxena

1. Attended Annual Meeting of Indian Association of Pathologist & Microbiologist (Delhi Chapter) held at RML Hospital on 5th April 2008.

2. Attended Specialty Board meeting of the Core Group experts organized by National Board of Examinations for the subject of Pathology held at NBE Campus, New Delhi on 1st June 2008.

3. Attended Project Review Committee meeting of NCD Division, ICMR held in ICMR on 5th-6th June 2008.

4. Attended World Cancer Congress-2008 held in Shanghai, China during 12th-17th June 2008 and presented paper "Differential gene expression in familial and tobacco associated esophageal cancers in north-east region of India".

5. Attended and presented paper "Study of molecular functional pathways associated with esophageal cancer in North-East India" for K.C. Mullick Award in APCON-2008 held in Chennai on 18th – 20th December 2008.

6. Attended Selection Committee Meeting for admission to DNB (Pathology) held in Safdarjang Hospital, New Delhi on 23rd July 2008.

7. Attended Scientific Advisory Group Meeting held in ICMR, New Delhi on 28th July 2008 and presented progress of scientific activities of Institute of Pathology during 2006 and 2007.

8. Attended Project Review Committee of North-East held in ICMR, New Delhi on 12th August 2008.

9. Invited for the Hundred Fifty First Meeting of the Senate of BITS, Pilani held on 22nd August 2008 at Birla Institute of Technology & Science, Pilani.

10. Attended Selection Committee Meeting for the selection of Assistant Professor (Pathology) held in Institute of Human Behavior & Allied Sciences, Delhi on 26th August 2008.

11. Chaired Ethical Committee Meeting held in Safdarjang Hospital, New Delhi on 27th August 2008, 20th January 2009 and 5th February 2009.

12. Attended Project Review Committee Meeting of NCD Division, ICMR, New Delhi on 10th September 2008.

13. Attended Expert Group Meeting on Breast Cancer held in ICMR, New Delhi on 18th September 2008 and presented progress of work in Task Force Project entitled "Establishment of Characterization of Cell Lines from Primary Breast Cancer".

14. Attended HUGO's 13th Human Genome Meeting, September 27 - 30, 2008, Hyderabad.

15. Attended Project Review Committee meeting of the BMS Division, ICMR on 17th October 2008.

16. Invited as Judge in the debate of English Society at "Montage-2008 Festival" at Jesus and Marry College, University of Delhi on the topic of "Religious Harmony is a myth" on 5th December 2008.

17. Delivered a talk on 'Prognostic and Predictive Factors in Cancer Management' at Foundation Day celebration & Workshop on Brachytherapy, Department of Radiotherapy at Convention Center, CSM Medical University, Lucknow on 14th December 2008.

18. Delivered a talk on "Breast cancer in Indian women : Risk and prevention" in 32nd Session of Indian – Social Science Congress (ISSC) held at Department of Biotechnology, Jamia Millia Islamia University, New Delhi on 18th December 2008.

19. Invited to deliver a talk on "Understanding molecular biology of cancer using Genomic approaches" at 63rd IAPM Kerala Chapter Meeing & 6th National CME in Pathology during 14-15th Feb. 2009 organised by Department of Pathology, Amrita Institute of Medical Sciences, Kochi.

20. Appointed Inspector by the National Board of Examination and inspected Department of Pathology, Sir H.N. Hospital, Mumbai for renewal of DNB accreditation on 5th January 2009.

21. Attended Technical Committee Meeting held in ICMR on 8th January 2009.

22. Attended the meeting of the Rastriya Arogya Nidhi held in the office of Director General Health Services, Nirman Bhawan, New Delhi on 20th January 2009.

23. Visited Dibrugarh for attending meeting with the collaborators of North-East Projects on 22nd - 23rd January 2009.

24. Attended Selection Committee meeting for the post of the Research Officer held at Institute of Pathology, New Delhi on 27th January 2009.

25. Attended Sub-Committee meeting of the Technical Committee held in ICMR on 29th January 2009.

26. Attended CME meeting held in All India Institute of Medical Sciences, New Delhi on 14th-15th February 2009.

27. Invited as Chief Guest to attend conference on "Emerging Trends in Life Sciences Research" organized by BITS, Pilani and delivered Key-note address on "Genome-wide approach to identify biomarkers for Esophageal cancer in North East India" on 6th March 2009.

28. Invited to attend Scientific Advisory Group Meeting of P&I Division, ICMR, New Delhi on 13th March 2009.

29. Invited to attend CDB-DBT Nasopharynx Project Meeting held on 14th March 2009 at Department of Biotechnology, New Delhi.

30. Attended the meeting of the Project Review Committee held in Jaipur on 18th – 19th March 2009 organized by N.C.D. Division, ICMR, New Delhi.

31. Attended the Mini Scientific Advisory Committee (Tumor Biology) meeting of the Institute of Pathology on 20th March 2009 held at Institute of Pathology, New Delhi.

32. Invited to attend Technical Committee meeting held at ICMR, New Delhi on 27th March 2009.

Dr. Aruna Singh

1. Attended 'Euro 2008 *Chlamydia* Meeting at Aarhus, Denmark from 1st July-4th July 2008.The topic of my talk was Determination of infectious load and immune parameters in asymptomatic, symptomatic and fertile women.

2. Invited to attend viva exam of a Ph.D student "Monoclonal antibodies to *Chlamydia trachomatis*-Characterization, sensitivity ,specificity and reactivity of hybrid clones in clinical samples" at Jiwaji University, Gwalior on 15th Sept. 2008.

3. Invited to attend one day dissemination workshop of IBBA-NH on 13th Sept. 2008

4. Invited to attend INSA Platinum jubilee celebrations on 10th Jan. 2009 and attended Mini symposium on "Indian science in global context" at New Delhi.

5. Invited speaker at International congress on " Bio-immunoregulatory mechanisms associated with reproductive organs : Relevance in fertility and Sexually transmitted infections" at National Institute of Immunology on Feb 9th -13th 2009. Topic of my talk was "Human mucosal immune response to *Chlamydia trachomatis* infection of the female reproductive tract".

6. Invited to deliver a key note address at National conference on Emerging trend in Life sciences Research at BITS, Pilani on March 6th, 2009. The topic of my talk was "Reproductive health of women and sexually transmitted diseases".

7. Invited to attend Workshop on commercialization of Biotechnology at India International Center, New Delhi on March 7, 2009

Dr. Sujala Kapur

1. Attended Chapter Meetings and Annual Conference of Pathologists and Microbiologists, Delhi Chapter, 2008-09.

2. Attended HUGO's 13th Human Genome Meeting, September 27 - 30, 2008, Hyderabad.

3. Attended Project Review meeting of Gastroenterology held at ICMR Headquarters, New Delhi (2008).

4. Attended HGM 2008 Satellite symposium on Clinical genomics organized by IGIB, New Delhi, 2008.

5. Attended Meeting with the Collaborators from Northeast India held at Dr B Barooah Cancer Institute, Guwahati, 2008.

6. Attended Meeting of Project Review Committee, Division of NCD, ICMR held at Guwahati, 2008.

7. Attended Meeting with the Collaborators from Northeast India at Dibrugarh, 2009.

8. Member, Selection Committee for Project Assistant at National Institute of Malaria Research (ICMR), Delhi, 2008.

9. Member, Departmental Promotional Committee for selection of Technical Officer, ICPO (ICMR), Noida, 2009.

Dr. Sangita Rastogi

1. Attended 8th Smt. Pushpa Sriramachari Foundation Day Oration on 'Protein chemistry and disease: The fusion of pathology and biochemistry' delivered by Prof. P. Balaram on 1st May 2008 at VMMC, Safdarjung hospital, New Delhi.

2. Guidance given to Senior Research Fellow in LSRB, DRDO project (2008).

3. Faculty member for 'WHO Basic and Advanced Level Training Courses for Laboratory Technicians' under auspices of Ministry of Health & Family Welfare at Institute of Pathology, New Delhi.

4. Compiled Performance budget document of IOP (July 2008).

5. Compiled Scientific information document of IOP (December 2008).

6. Examiner for M.Sc. IIIrd Semester Practical examination of CCSU (February 2009).

7. Attended International Congress on 'Bioimmunoregulatory mechanisms associated with reproductive organs: Relevance in fertility and in sexually transmitted infections' on 9^{th} -13^{th} February 2009 at National Institute of Immunology, New Delhi.

8. Participated in 25th Annual conference of ISOPARB-2009 on 21^{st} -22^{nd} March 2009 at India Habitat Center, New Delhi.

Dr. Poonam Salotra

1. Elected Fellow of the National Academy of Sciences, India, in the year 2008.

2. Appointed Associate Editor of BMC Infectious diseases in 2009.

3. Invited by WHO as member of Expert Committee for Leishmaniasis control, TDR- WHO in 2009.

4. Appointed reviewer for several project proposals submitted to DBT, DST, ICMR.

5. Appointed external examiner for Ph.D. thesis evaluation and viva by BHU, Varanasi in April 2008 and by CDRI, Lucknow in Dec 2008.

6. Chaired the session on "Drug resistance and mechanism" at World Leishmania Congress IV and presented work entitled "Drug resistance in field isolates of visceral leishmaniasis: The Indian perspective" in Feb. 2009.

7. Invited speaker in symposium on "Diagnostics and test of cure for visceral leishmaniasis" organized by Infectious Disease Research Institute, Seattle, USA at World Leishmania Congress IV held at Lucknow in Feb. 2009.

Dr. AK Jain

1. Invited to chair a scientific session in National Conference on Electron Microscopy held at Bundelkhand University, Jhansi from January 17 to 20, 2009.

2. Conducted WHO-sponsored Basic level and Advanced level training courses for technicians at Institute of Pathology from February 16 to March 14, 2009.

3. Helped the Director in the organization of Pushpa Sriramachari Foundation Day Oration delivered by Prof. P. Balaram on 1st May 2008.

4. Participated in Pre-Ph.D. programme of BITS Pilani.

5. M.Sc. Dissertation supervised**:**
   i) Ultrastructural, immunohistochemical and trace element studies of placenta in Indian women with IUGR deliveries. For the award of M.Sc. (Biotechnology). Jiwaji University, Gwalior. July 2008.

6. Meet on Open Access, FOSS and Copyright Law for Scholarly communication and Literary Work on 26th April 2008 at Indian National Science Academy, Bahadur Shah Zafar Marg, New Delhi.

7. Forty Fourth Founder Memorial Lecture of Shriram Institute for Industrial Research, New Delhi entitled "Radiation Processing" delivered by Dr. Anil Kakodkar, Chairman, Atomic Energy Commission and Secretary Deptt. Of Atomic Energy.

8. Eighth Smt. Pushpa Sriramachari Foundation Day Oration entitled "Protein Chemistry and Disease: The Fusion of Pathology and Biochemistry" delivered by Prof. P. Balaram, Director Indian Institute of Science at Vardhman Mahavir Medical College, New Delhi on 1st May 2008.

9. Attended lecture on Homogenization by Prof. S. R. S. Varadhan, Raman Professor, Indian Academy of Science (Courant Institute of Mathematical Science, New York) at Indian Statistical Institute, Delhi on 4th Feb. 2009.

10. Paper Presented on Electron Microscopy as a tool for characterization of Nanomaterials, polymers and Bio-organic molecules**:** Invited Talk during ISAS – DC Seminar held at Malviya Hall, Lucknow University, Lucknow on 2nd August 2008.

11. Papers Presented on Electron Microscopy for Biologists**:** Invited talk during CEP course conducted by DRDE, Gwalior in Nov. 2008.

12. Paper Presented on Role of Electron Microscopy in Investigative and Diagnostic Dermatology during National Conference on Electron Microscopy and XXX Annual meeting of Electron Microscope Society of India, Bundelkhand University, Jhansi January 17-20, 2009.

13. Paper Presented on Electron Microscopic and Vascular Endothelial Growth Factor Receptor – 1 in Pre-Eclampsia patients during National Conference on Electron Microscopy and XXX Annual meeting of Electron Microscope Society of India, Bundelkhand University, Jhansi January 17-20, 2009.

14. Paper Presented on Studies on ultrastructural changes of placenta in patients having IUGR deliveries during National Conference on Electron Microscopy and XXX Annual meeting of Electron Microscope Society of India, Bundelkhand University, Jhansi January 17-20, 2009.

15. Paper Presented on Ultrastructural studies of sperm in idiopathic male infertility during National Conference on Electron Microscopy and XXX Annual meeting of Electron Microscope Society of India, Bundelkhand University, Jhansi January 17-20, 2009.

16. Paper Presented on Ultrastructural studies in mice tail model of psoriasis during National Conference on Electron Microscopy and XXX Annual meeting of Electron Microscope Society of India, Bundelkhand University, Jhansi January 17-20, 2009.

17. Paper Presented on An Ultrastructural study of the umbilical cord tissue in low birth weight babies during National Conference on Electron Microscopy and XXX Annual meeting of Electron Microscope Society of India, Bundelkhand University, Jhansi January 17-20, 2009.

18. Paper Presented on Studies on use of different catalysts during processing for TEM during National Conference on Electron Microscopy and XXX Annual meeting of Electron Microscope Society of India, Bundelkhand University, Jhansi January 17-20, 2009.

19. Paper Presented on Apoptosis in Molar Pregnancy during National Conference on Electron Microscopy and XXX Annual meeting of Electron Microscope Society of India, Bundelkhand University, Jhansi January 17-20, 2009.

Dr. AK Bagga

1. Won third award In 'Vaad-Vivad Pratiyogita' held at ICMR Headquarters. The topic of Pratiyogita was "Bhart Mein Chikitsiya Parikshanon Mein Nitivishayak Pahaluon Ka Anupalan" on 25-09-2008.

2. Attended the Quarterly Meeting of Delhi Chapter of Indian Association of Pathologists & Microbiologists at Auditorium, PGIMER Complex, Dr. Ram Manohar Lohia Hospital, New Delhi on 30-07-2008.

Dr. Usha Agarwal

1. Deputed to participate in the core committee meeting for "Application of Technology in Diagnostic Pathology" on 29th July 2008 at Deptt. of Pathology, AIIMS.

2. Co-ordinated the DNB Inspection by Dr Rajalakshmi, Institute of Child Health, Egmore, Chennai for extension of accreditation of the Institute of Pathology for conducting DNB courses.

3. Faculty for WHO Basic and Advanced Technicians Training Programmes held from 16th February to 15th March 2009.

4. Faculty for PhD students of Institute of Pathology, ICMR, New Delhi registered with BITS, Pilani.

5. Served as Expert on the Selection Committee for the posts of DNB students on 18th Feb. 2009.

6. Served as expert in interviews for Lab. technicians in Patel Chest Institute.

Dr. Lakshman Kr. Yerneni

1. Teaching Faculty in Training course entitled "Basic Techniques in Stem Cell Biology: Isolation, Maintenance and Differentiation" held at CCMB, Hyderabad from 25th February to 10th March 2008.

2. Invited to deliver a talk entitled "Ins And Outs Of Reconstructed Skin Substitutes For Wound Healing" as an Invited speaker during a Symposium entitled "Trends in Regenerative and Molecular Medicine" to be held at the Centre for Genetic Diseases and Molecular Biology, Department of Biochemistry, Pt.JNM Medical College, Raipur on 25th July 2008.

Dr. Saurabh Verma

Supervisor:

1. M.Sc. (Biochemistry) dissertation titled, "Flow Cytometric study of surface antigen markers (CD3, CD4 and CD8) and DNA content analysis in breast cancer patients by Mr. Rishikesh Shukla from Jiwaji Universtity, Gwalior, 2007.

2. M.Sc. (Biochemistry)dissertation titled, "Flow cytometric study of Cox-1 and Cox-2 Expression in correlation with cytokines dysfunction in Transitional Cell Carcinoma (TCC) patients by Ms. Swarna Saxena from Jiwaji Universtity, Gwalior, 2008.

Dr. Purnima Paliwal

1. Attended the 14th Indo-US International CME in surgical Pathology, Cytology and Hematology held at Agra wef 3rd to 5th February 2009.

2. Attended IAPM (Delhi Chapter) quarterly and annual meets.

Dr. AK Mishra

1. Attended '56th International Statistical Institute Conference' at Lisboa-Portugal from 22nd August 2007 to 29th August 2007 and presented the research paper entitled "Some Epidemiological Models for the Parasitic Infections: Comparison between Traditional and Hierarchical Logistic Regression Methods".

2. Attended five day workshop on 'Bioinformatics' at the Maulana Azad National Institute of Technology (MANIT), organized under the infrastructure facility of the Department of Biotechnology (DST), in Bhopal from 10th-14th March 2008.

3. Attended one day training workshop on Classification and Segmentation techniques using SPSS 16.0 at Institute for Integrated Learning and Management (IILM), New Delhi, on 28th February 2008.

4. Attended one day workshop on Integration of Microbicides into Social Sciences Research organized by Family Health International a division of National Institute of Health (NIH), USA, at Hyatt, New Delhi on 23rd February 2008.

5. Attended three day conference of 'First Indo US Summit' organized by American Association of Physicians of Indian Origins (APPI) in association with Indian Medical Association (IMA), Medical Council of India (M.C.I.) and Government of India at Hyatt, New Delhi from 13th-15th December 2007.

6. Attended 29th National Conference of the "Indian Association for the Study of Population and National Seminar on Recent Statistical Technique for Data Analysis" at Banaras Hindu University (B.H.U.), Varanasi, 26th – 28th October, 2007 and presented paper entitled "Statistical Considerations in Breast carcinoma-A Study on Association of Androgen Receptors with Clinical Response".

Teaching

1. Involved in the teaching of "Biostatistics and Biomodelling" under the Off-distance Campus Programme' in collaboration with BITS, Pilani, to students as part of their 'Pre Ph.D Qualifying' examination for the year 2007.

2. Delivered a presentation on "Fundamental Aspects of Statistics and Biostatistics" in the journal club as a part of the academic activities of the institute.

SCIENTIFIC ACTIVITIES / SEMINARS/WORKSHOPS AT INSTITUTE OF PATHOLOGY

1. **9th Smt Pushpa Sriramachari** Oration Award Lecture by **Dr. P Balaram Director, Indian Institute of Science, Bangalore** at Institute of Pathology, New Delhi on 1st May 2008.

2. Inspection for renewal of **accreditation of DNB program** at IOP. **Dr. Rajalakshmi, Prof. Of Pathology**, Institute of Child Health, Egmore, Chennai was Inspector of National Board of Examination on 4th Aug. 2008.

3. Guest lecture by **Dr. Caryn Bern,** Centre for Disease Control, Atlanta, Georgia, USA on "**The epidemiology of visceral leishmaniasis and PKDLs with a focus on recent studies from Bangladesh**" on 4th Sept. 2008.

4. Guest lecture by **Dr. Madhuri Karkarala**, Univ. Hospital of Michigan, MI, USA on **"Curcumin, piperine and curcumin dipiperoyl in breast cancer prevention**" on 12th Nov. 2008.

5. Guest lecture by **Dr. Francis O. Eko**, PhD, Asso. Prof., Deptt. of Microbiology, Biochemistry and Immunology, Morehouse School of Medicine, Atlanta, Georgia, USA on "**Novel strategies in vaccine development**" on 24th Nov. 2008.

6. Guest lecture by **Dr. Bernard P Arulanandam**, Prof. of Microbiology and Immunology, Deptt. of Biology, Univ. of Texas, San Antonio, Texas on "**New insights into protective immune mechanisms against chlamydial STD**" on 10th Feb. 2009.

7. Guest lecture **by Dr. Anindya Dutta**, Prof. of Biochemistry & Molecular Genetics, Univ. of Virginia Health Sciences Center, Charlottesville, VA on "**Micro RNAs in differentiation and cancer**" on 13th Feb. 2009.

8. Pre- Sac Meeting on Tumor Biology/ Cell Biology/ Infectious Diseases / Environmental Biology was held on 17th March and 20th March at IOP.

9. Guest lecture by **Prof RN Saha**, Prof of Pharmacy, Dean, Faculty Division III and Educational Development Division, BITS, Pilani (Rajasthan) on "**Some studies on nonparticulate drug delivery systems for selected anti-cancer drugs**" on 30th March 2009.

10. Students of M.Sc. (Biotechnology) and faculty members of Nehru Arts and Science College, Coimbatore visited IOP on 18th July 2008.

11. Independence Day was celebrated at IOP on 14th Aug. 2007.

12. Guest lecture by **Mr. Kenneth Harris**, Chairman, M/S Totipotent SC Scientific Product Pvt. Ltd., Chennai on "**Ex-vivo cell therapy processing system vis-à-vis conventional clean-room approach**" on 18th Nov. 2008.

13. Guest lecture by **Dr. SK Kaul**, Scientist F, Solid State Physics Laboratory, Delhi on "**Potential clinical applications of stem cells in burns**" on 19th Nov. 2008.

14. **WHO In-country Fellowship** Training Programme for **Basic Level and Advance Level Technicians** conducted from 16th Feb.2009 to 14th March 2009.

DNB PROGRAMME

The Post-Graduate Level Training Programme in the speciality of Pathology continued during 2007-08. During the year, **two** students were admitted in the DNB course at Institute of Pathology:

1. **Dr. Disha Arora**
2. **Dr. Ila Jain**

The following two students who appeared for the DNB Theory Exam held in February/March 2008 have come out with flying colours:

1. **Dr. Punita Rao**
2. **Dr. Monika Dixit**

As per guidelines of the National Board of Examination, the Institute conducted Review Examinations of the DNB students in July 2008. Dr. Amit Kumar Dinda, Additional Professor, Department of Pathology, AIIMS, New Delhi came as reviewer.

Ph.D Programme

1. Ms. G. Srividya was conferred with the degree of Doctorate of Philosophy for her work on Leishmaniaisis by BITS-Pilani in Nov. 2008.

2. Mr. Rishein Gupta submitted his Ph.D theis on Chlamydiasis to BITS-Pilani in March 2009.

3. Mr Rajesh Kumar submitted his PhD thesis on Leishmaniasis to BITS-Pilani in Jan. 09**.**

4. 21 students are currently registered for the Off-Campus Ph.D. programme of BITS-Pilani.

5. 7 students are currently registered for PhD programme of **Jiwaji University** (Gwalior), **Ch. Charan Singh University** (Meerut) and **I.P University** (Delhi), **Jamia Millia University** (New Delhi), **Jamia Hamdard University** (New Delhi).

During 2008-09, Institute of Pathology further attracted young researchers-research scholars with CSIR Junior Research Fellowships (6), ICMR Senior Research Fellowships (10) and UGC Fellowships (6) joined the Institute during this period.

TRAINING PROGRAMME

Under the Training Programme, the following **six** students deputed by **Jiwaji University, Gwalior** underwent research training for doing six-months project work in different laboratories at Institute of Pathology:

1. Ms. Thangyam Jeena Devi
2. Ms. Ankur Saxena
3. Mr. Ratnam Prasad
4. Mr. Urja Jaiswal
5. Ms. Sonia
6. Ms. Priyanka

Subsequent to completion of their project work, the students will submit their dissertations.

OTHER ACADEMIC ACTIVITIES

As part of academic activities, the Institute organized journal clubs, slide seminars and seminars by various experts from both within and outside the country.

Scientific activities of Ph.D./DNB students

Bharat Bhushan

1. NF-kB signaling pathway in acute leukemia: A study on expression of cell survival and proliferative genes by Real Time RT-PCR. Bhushan B, Chauhan P S, Saluja S, Mishra A K, Bhasin S, Gupta D K, Siddiqui S, Kapur S. Proceedings of 13th Human Genome Meeting (HGM 2008), Hyderabad, 2008.

2. Attended the international conference organized by European School of Oncology (Inside Track Conference) on "Leukaemias: Molecular Insights to Treatment Paradigms" held on 7th – 9th March 2008 at Mumbai, India.

Pradeep Singh Chauhan

1. Presented Poster on "Expression of Ki67 as a prognostic indiacator in acute leukemia" in National Conference on Emerging Trends in Life Sciences Research held on March 6th-7th, 2009 at BITS, Pilani , Rajasthan.

2. Attended the Fourth Workshop on "Genetic Epidemiological Methods for Dissection of Complex Human Traits" held from Feb. 23-28, 2009 organized by TCG-ISI Centre for Population genomics (CpG), Kolkatta.

3. Presented Poster on "Immunophenotypic and clinical findings in adult acute myeloid leukemia with FLT3 internal tandem duplication" in HUGO's 13th Human Genome Meeting, September 27 - 30, 2008, Hyderabad.

Mishi Kaushal

1. Poster Presented on "Significance of TP53 codon 72 polymorphism in lung and breast cancer showing different xenobiotic potential" in Thirteenth Human Genome Meeting (HUGO) 2008, Hyderabad.

2. Attended the Fourth Workshop on "Genetic Epidemiological Methods for Dissection of Complex Human Traits" held from Feb. 23-28, 2009 organized by TCG-ISI Centre for Population genomics (CpG), Kolkatta.

3. Presented Poster on "Genotype polymorphism in xenobiotic metabolizing genes and their contribution in breast cancer susceptibility in Northeast Indian population" in National Conference on Emerging Trends in Life Sciences Research held on March 6th-7th, 2009 at BITS, Pilani, Rajasthan.

Dhirendra Singh Yadav

1. Poster entitled "Genetic polymorphisms of CYP1A1 genotypes in various ethnic groups of India" presented in "International Symposium on Ethics Culture and Population Genomics & 34th Annual Conference of the Indian Society of Human Genetics" organized by ISHG and Anthropological Survey of India, in New Delhi on March 17-20, 2009.
2. Gave an Oral presentation on "Genetic Polymorphisms of CYP1A1 genotypes in patients with oral cancer" in "National Conference On Emerging Trends In Life Sciences Research" organized by BITS Pilani in Pilani on March 6-7, 2009.
3. Presented poster entitled "Prognostic value of TP53 Codon 72 polymorphism in oral cancer and stomach cancer in high risk region of India" in 13th Human genome meeting (HGM 2008) entitled "Genomics and the Future of Medicine" organized by CSIR and HUGO international meetings held in Hyderabad on September 27-30 2008.

Thoudam Regina

1. Presented poster entitled 'Differential gene expression profile of stomach and oral cancer in high risk region of India'. Human Genome Meeting 2008, Hyderabad Regina D Thoudam, Dhirendra S Yadav, I Chattopadhyay, AC Kataki, E Zamoawia, S Kapur, S Saxena.

2. Attended the Fourth Workshop on Genetic Epidemiological Methods for Dissection of Complex Human Traits held from Feb. 23-28, 2009 organized by TCG-ISI Centre for Population Genomics (CpG), Kolkatta.

3. Presented poster entitled "Detoxifying enzyme genotypes and susceptibility to gastric cancer. National Conference on Emerging Trends in Life Sciences Research. Th. Regina Devi, D.S. Yadav, A.C. Kataki, E. Zamoawia, Y. Verma, S. Kapur, S. Saxena. March 6-7, 2009 at Birla Institute of Technology & Science, Pilani-333031, Rajasthan.

4. Presented poster entitled 'Polymorphisms of Tumour Protein P53 genes and the risk of developing Gastric Cancer in North East India. Intenational Symposium on Ethics, Culture and Population Genomics' & 34th Annual Conference of the Indian Society of Human Genetics 2009. Th. Regina Devi, D.S. Yadav, A.C. Kataki, E. Zamoawia, Yogesh Verma, S. Kapur, S. Saxena.

Rakhshan Ihsan

1. Presented Poster on "Influence of Germline Polymorphisms of TP53, GSTM1 and GSTT1 Genes in Lung Cancer Risk" in National Conference on Emerging Trends in Life Sciences Research held on March 6th-7th, 2009 at BITS, Pilani , Rajasthan.

2. Attended the Fourth Workshop on Genetic Epidemiological Methods for Dissection of Complex Human Traits held from Feb 23-28, 2009 organized by TCG-ISI Centre for Population genomics (CpG), Kolkatta.

3. Presented Poster on "Significance of TP53 codon 72 polymorphism in breast and lung cancer showing different xenobiotic potential spectrum" in HUGO's 13th Human Genome Meeting, September 27 - 30, 2008, Hyderabad.

Indranil Chatterjee

1. Presented Poster on "Differential expression of MAPK and GPCR pathway in esophageal cancer of North-east region of India." in Human Genome meeting (HGM 2008 Workshops), Hyderabad, September 27-30, 2008.