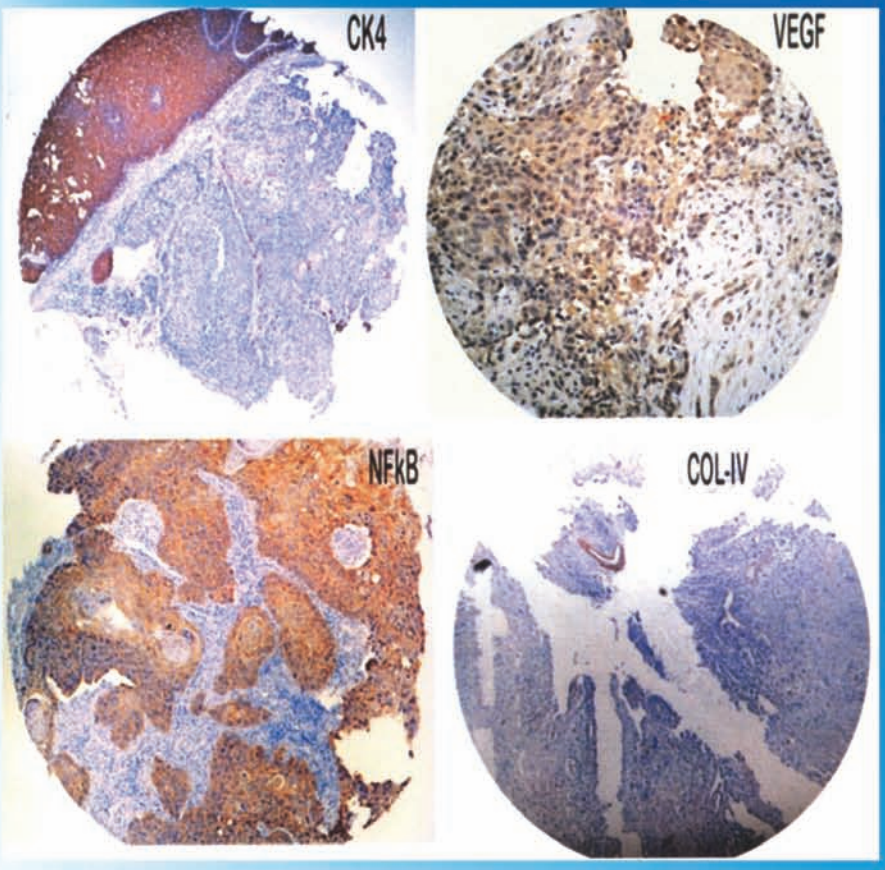
ANNUAL REPORT

2007-2009



INSTITUTE OF PATHOLOGY (ICMR)

SAFDARJANG HOSPITAL CAMPUS, NEW DELHI

Photomicrograph of tissue microarray cores from esophageal tumor biopsies obtained from familial ESCC patients showing negative immunostaining for KRT4 (A) and collagen IV (D) and positive immunostaining for VEGF (B) and NF-$_{\kappa}$B (C).

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INSTITUTE OF PATHOLOGY
SAFDARJANG HOSPITAL CAMPUS
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CONTENTS

EXECUTIVE SUMMARY

It gives me immense pride in presenting the scientific achievements and academic activities at Institute of Pathology during the year 2007-08 and 2008-2009. 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 for better management. The main focus for research activities has been cancer biology, infectious diseases mainly *Leishmania* and *Chlamydia*, stem cell biology and environmental toxicology. Human resource development has been an integral activity at IOP.

To identify the women at high risk of developing breast cancer, a candidate gene approach had been used to identify the genetic alterations constituting high risk profile. The CYP17 A2 allele, VDR Poly A L allele and ≥20 CAG repeats in AR genes were categorized as putative high risk alleles and women carrying genotype with three putative high risk alleles were found to have increased risk of developing breast cancer (OR=4.68) than those carrying one or two putative high risk alleles. In order to identify profile of high risk genes at large scale, a study of genome wide scanning has been initiated to study the gene expression and hypermethylation profile in early and late onset breast cancer. One breast cancer cell line 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. Correlation of expression of type 1 growth factor receptor genes EGFR, c-erbB-2, c-erbB-3 and 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 microarray has been completed. Pathways found significantly upregulated in both tobacco associated and familial cancers include MAPK pathway, neuroactive ligand receptor interaction, calcium signaling pathways and down regulated pathways include extra-cellular matrix organization, structural constituent of ribosome, cell communication and apoptosis activity. Beside, 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 microarray 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 microarray 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 associations of bcl-IGH translocations and GST gene polymorphisms were 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 chemotherapy 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 microarray (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 microarray at National Cancer Institute for analysis of potential diagnostic and prognostic biomarkers using immunohistochemistry (TMA-IHC).

Initial studies on chlamydial infection were mainly on genital infection but recently study has been extended to coronary artery disease also. Study on role of chlamydial 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 with women with 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 *C. trachomatis*, expression of transferrin receptor (TfR) was found down-regulated; whereas expression of ferritin heavy chain (FHC) was up-regulated in *C. trachomatis* infected HeLa 229 cells. Expression of TfR in infected cells did not change upon addition of iron chelatar 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.

In studies on leishmanial infection, global variation in gene expression was studied in promastigotes, an intermediate stage of differentiation (PA24) and axenic amastigotes in culture by using genomic microarrays to understand process of parasite differentiation and to identify virulence related genes. 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-β) 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 complex network of effecter/regulatory interactions. Analysis 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 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. Studies on Multilocus Microsatellite Typing (Mlmt) reveal 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 undergone major building renovation activities occupying bulk time of scientists but the annual report depicts 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. I congratulate **Dr. Poonam Salotra** for receiving **Prof. BK Aiket Oration Award for year 2004** and **Basanti Devi Amir Chand award for year 2006** by Indian Council of Medical Research. She was also elected **Fellow of the National Academy of Sciences**, India. **Dr. Sunita Saxena** received **K.C. Basu Mullik Award for 2008** from **Indian Association of Pathologists and Microbiologists** and **Dr. AP Singh** was awarded **Indo-US research fellowship** to work at Advanced Technology center, Tissue Array Research Lab, National Cancer Institute, NIH. **Mr. Indranil Chatterji**, SRF and Ph.D student was awarded **ICRET fellowship of UICC**. 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

RESEARCH ACTIVITIES

TUMOR BIOLOGY

TUMOR BIOLOGY

A. BREAST CANCER

1. STUDY OF CANDIDATE GENES ASSOCIATED WITH BREAST CANCER SUSCEPTIBILITY

Scientific Staff	:	Dr. Sunita Saxena, Anurupa Chakraborty
Collaborators	:	Dr. D. Bhatnagar, Dr. Chintamani, Dr. Mohil, Dr. A. Bhatnagar, Safdarjang hospital
Technical Staff	:	Mrs. Valsamma Mathew
Duration	:	2004-2008

Aims, Objectives and Background

Breast cancer constitutes the most common malignancy and the most common cause of cancer related deaths in Indian women. Common and rare low-moderate penetrance genes and high-penetrance genes are thought to explain the genetic susceptibility to the disease. Individuals have different susceptibility towards environmental exposures that may cause or contribute to breast cancer and this inter-individual variation is partly due to genetic polymorphisms in genes involved in DNA repair, which have potential to modulate the function of encoded proteins or the genes responsive to endogenous hormones since endogenous steroid hormone exposure is known to influence breast cancer risk.

This project is a contribution to the knowledge about the importance of mutations and polymorphisms and how they might interfere with the health. More specifically, the aims have been to investigate the distribution and the nature of BRCA1 and BRCA2 germline mutations and polymorphisms in a cohort of North-Indian breast cancer patients and to assess the possible association of CYP17, Vitamin D Receptor and Androgen Receptor gene with breast cancer risk and to develop a multigenic model of breast cancer susceptibility.

Work done during the period

In total, 18 genetic alterations were identified in *BRCA1* and *BRCA2* genes following screening of 204 breast cancer patients. Of these, six were pathogenic protein truncating mutations. Three deleterious frame-shift mutations were identified in *BRCA1*, along with one missense mutation, one 5'UTR alteration, three intronic variants and one silent substitution. Similarly, three pathogenic protein-truncating mutations along with one missense mutation, four intronic alterations and one silent substitution were identified in BRCA2. Mutations were found distributed throughout the genes and some of them are novel to Indian patients. These mutations were found more prevalent among early-onset cases of Indian women compared to late-onset and familial breast cancer patients.

The study further suggests that there exists a possible correlation between CYP17 genotype and breast cancer and the polymorphism in the same gene influences early-onset breast cancer by increasing the risk of disease in individuals carrying the A2 allele. This provides an important early step in defining model that can explain individual susceptibility in terms of genetic susceptibility and endogenous estrogen exposure. Although, *Apa*I and *Taq*I did not show any significant association with the disease when analyzed in isolation, however they might modulate the risk associated with (L) alleles of VDR Poly-A which showed significant association with breast cancer risk in North-Indians. Further, individuals with 20 or more than 20 AR-CAG repeat units were found more prone to develop breast cancer (P= 0.09). When case subjects were classified according to number of high-risk alleles involved in hormonal pathways, the data revealed a trend of increasing breast cancer risk with increase in number of high risk alleles. These findings suggest that breast cancer risk has a strong genetic component and supports the theory that the underlying mechanism of "complex traits" can be understood using a multigenic model of low penetrant genes. This study has been concluded.

2. ESTABLISHMENT AND CHARACTERIZATION OF BREAST CANCER CELL LINES FROM PRIMARY BREAST CANCERS

Scientific Staff	:	Dr. Sunita Saxena, Dr. S. A. Raju Bagadi, Dr. Sujala Kapur, Shanti latha P.
Collaborators	:	Dr. D. Bhatnagar, Consultant, Dr. Chintamani, Dr. R. S. Mohil, Dr. A. Bhatnagar, Safdarjang hospital
Technical Staff	:	Mr. Jagdish Pant
Duration	:	2007-2010

Aims, Objectives and Background

Although the incidence of breast cancer in India is low as compared to the western countries, the incidence of breast cancer in younger women remains more or less same suggesting a high proportion of early onset of breast cancer in Indian women. The molecular mechanism associated with early breast cancer is not well understood. Therefore, there is a need to establish cell lines from Indian population, which may be useful for understanding molecular mechanisms involved in breast tumourigenesis in Indian population. Cell lines provide an unlimited, self-replicating source of cells that can be widely distributed to facilitate comparative studies. The majority of breast carcinoma cell lines have been established from tumour metastases, while relatively few have been established from primary tumours. Thus, the present study is aimed to develop breast cancer cell line from primary breast tumours that may serve as a good *in vitro* model for understanding early breast tumourigenesis and mechanisms underlying breast tumourigenesis in Indian population. The objectives of the project include: a. Establishment of cell line(s) from human primary breast tumours, b. Characterisation of the established breast cancer cell lines.

Work done during the period

During the period under report, 43 breast tumours were collected and used for initiation of primary cultures. Primary cultures were established by enzymatic disaggregation (using trypsin and collagenase). Twenty five primary cultures could be established using the 43 patient samples, of which 9 cultures are currently growing in laboratory and passed more than 5 passages. All the primary cultures established were subjected to purification by differential

trypsinsation and or Magnetic Activated Cell Sorting (MACS) using Cytokeratin or EpCAM antibodies. The purified cultures were characterized for expression of epithelial markers such as Epithelial Membrane Antigen (EMA), Cytokeratins (CKs); mesenchymal marker, vimentin; estrogen recetptor α (Figure 2). Among the cultures currently growing in the laboratory, three cultures PCB20 (p35), PCB12 (p14), PCB17 (p15) have crossed more than 10 passages, as PCB20 has crossed more than 30 passages this culture has been considered as established continuous cell line, further this culture is a rapidly growing homogenous epithelial line (Fig.1). PCB20 is positive for EMA and CK and is ER negative (Fig.2). PCB36 is another potential cell line, which is rapidly growing and homogenous epithelial culture. Further, anchorage independent growth of these cultures was assessed on soft agar, anchorage independent growth was shown by PCB12, BCB20, PCB33 (Fig. 2) on soft agar. Characterization in terms of ploidy, growth characteristics, *in vivo* tumorigenisity is currently underway for these cultures.

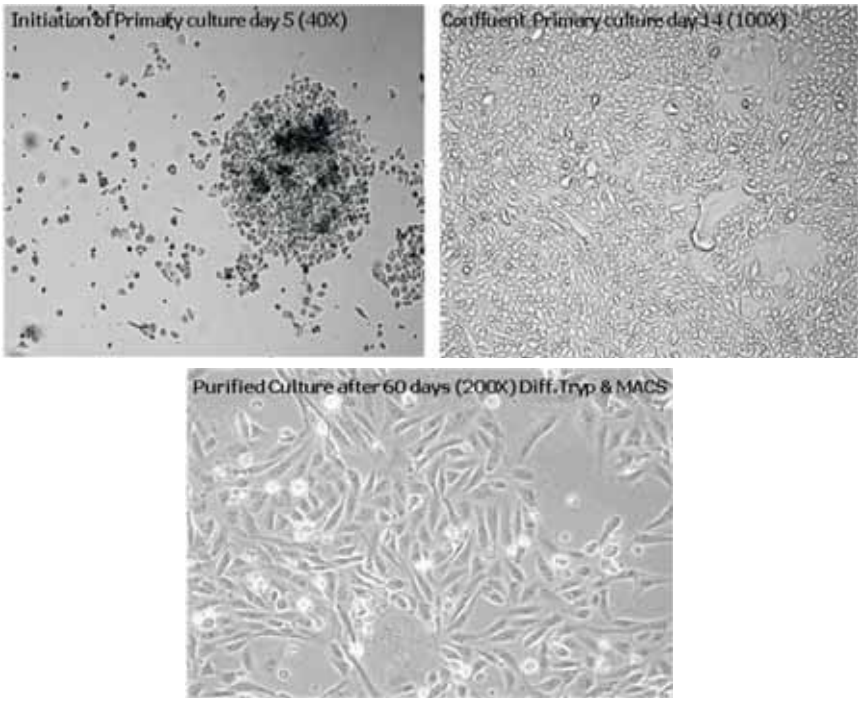


Fig. 1: Establishment and Purification of PCB20

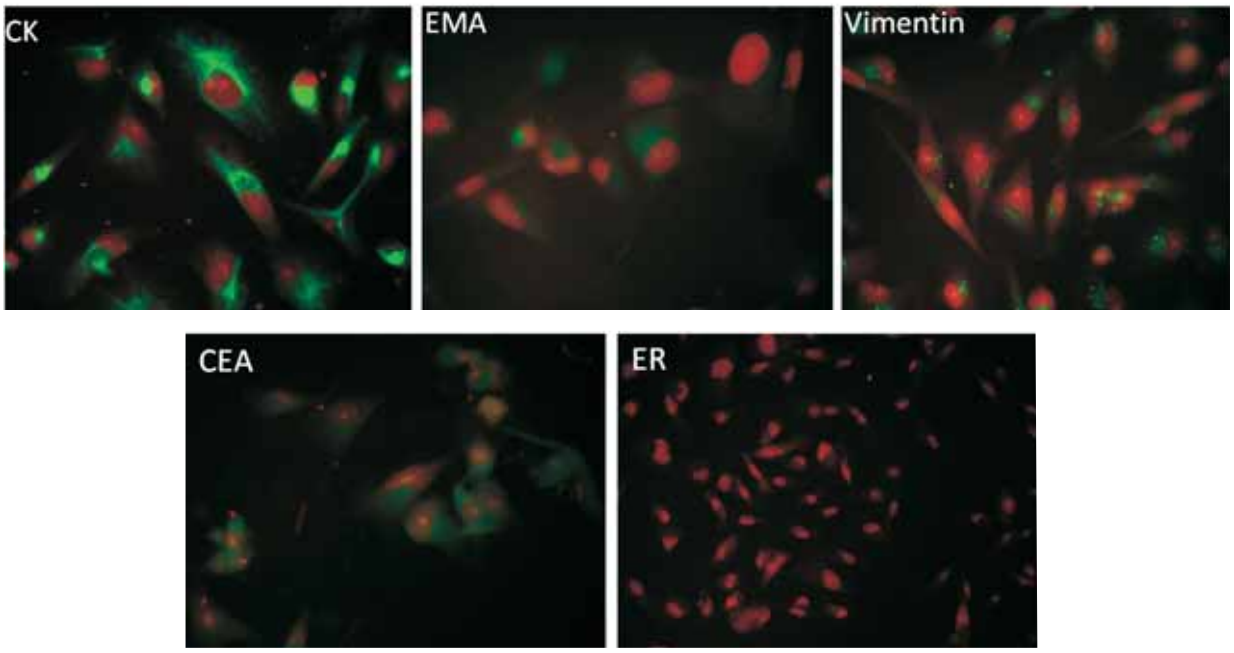


Fig. 2: Expression of Biological Markers in PCB 20 Cell Line

3. STUDY ON GENE EXPRESSION AND HYPERMETHYLATION PROFILES IN EARLY ONSET BREAST CANCER

Scientific Staff	:	Dr. Sunita Saxena, Dr. S. A. Raju Bagadi, Dr. Sujala Kapur, Macha
Collaborators	:	Dr. D. Bhatnagar, Dr. Chintamani, Dr. R. S. Mohil, Dr. A. Bhatnagar, Department of Surgery, Safdarjang hospital
Technical Staff	:	Mrs. Valsamma Mathew
Duration	:	2008-2011

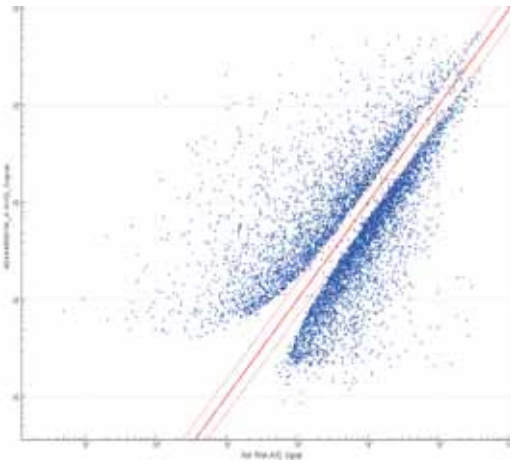
Aims, Objectives & Background

Breast cancer is the second leading cause of cancer deaths among women globally and is the most prevalent cancer in the world with 4.4 million survivors up to five years after diagnosis. It is the second most common cancer among Indian women, after cancer of the cervix uteri. Even though the incidence of breast cancer in India is low as compared to the western countries, the incidence of breast cancer in younger women remains more or less same suggesting a

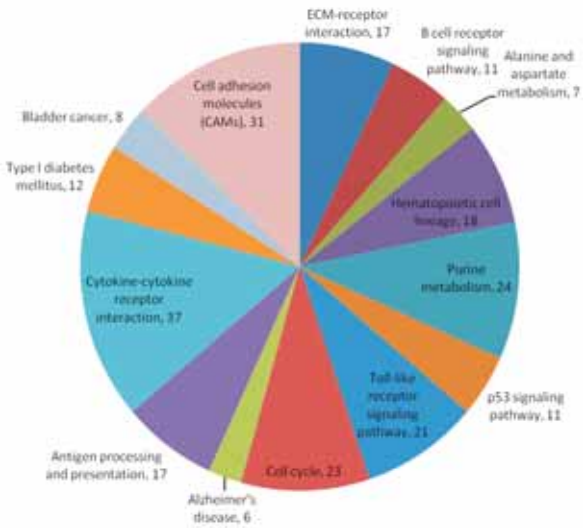
high proportion of early onset breast cancer in Indian women. The molecular mechanism associated with early breast cancer is not well understood. Analyzing molecular signatures associated with early onset breast cancer may provide insight into the associated molecular mechanisms. Hence we wanted to study the gene expression and methylation profiles of early onset breast cancer using microarray technology, which we hope will shed light on various pathways involved in tumorigenesis. Thus the present study is aimed to elucidate genetic and epigenetic factors associated with early onset breast cancer in Indian women, and the objectives include: a. To study differential gene expression profile in early onset breast using microarray technology, b. To study promoter methylation changes associated with early onset breast cancer.

Work done during the period

In the year under report, 38 cases of breast cancer have been collected, which include: 15 mastectomy, 18 excision, and 6 trucut biopsies. The age of patents ranged from 35-87 years, among these 9 cases are of early onset (<40 years) and 20 are of late onset breast cancers (>55 years). Patients between 40-55 years were excluded from the study. RNA and DNA have been isolated from all the samples collected. Before proceeding for microarray experiments, RNA quality was assessed further on Bioanalyzer (Agilent). RNA from 10 breast cancer specimens were selected based on their quality and used for gene expression profiling. RNA from adjacent normal specimens were not of adequate quality and quantity for microarray experiments, hence Universal Human Reference RNA (from Stratagene) was used as control in these experiments. RNA was labeled and hybridized to the slides and the hybridised slides were scanned on micro-array scanner and data was acquired. The data has been average normalized, gene expression changes with respect to universal Reference RNA were calculated and gene list was generated on the Bead array studio (Illumina). Test of significance ('t' tests) ($P < 0.05$) and differential score (+13 /-13) provided by the Bead Studio software were used to identify genes that are differentially expressed (Fig. 3a). Genes with expression >2.0 fold were considered as over-expressed, genes with expression < 2 fold were considered as under-expressed. About 1800 differentially expressed genes belonging to various pathways were obtained upon initial bioinformatics analysis (Fig. 3b), however, detailed analysis will be done after doing microarray analysis in more number of samples.



A



B

Fig. 3a: Scatter plot showing differential expressed genes with respect to universal human reference RNA.
Fig. 3b: Clasification of upregulated genes into various pathways.

4. TYPE 1 GROWTH FACTOR RECEPTOR FAMILY : EXPRESSION AND CORRELATION WITH RESPONSE TO NEO-ADJUVANT CHEMOTHERAPY IN LOCALLY ADVANCED BREAST CANCER

Scientific Staff	:	Dr. Sunita Saxena, Dr. L.C. Singh, Dr. Sujala Kapur
Collaborators	:	Dr. Dinesh Bhatnagar, Dr. Chintamani, Dr. Nidhi Sugandhi, Safdarjang hospital
Duration	:	2006-2009

Aims, Objectives and Background

Locally advanced breast cancers are advanced-stage nonmetastatic breast tumors and include a wide variety of clinical scenarios (T3N1; Any T4, Any N2, N3, M0). These tumors remain a difficult clinical problem as most patients with locally advanced disease will experience

disease relapse and eventual death. Neoadjuvant chemotherapy with three cycles of CMF (Cyclophosphamide, methotrexate, 5-flourouracil) are given to the patient to downstage tumors, with aim of rendering them more amenable to conservative surgery.

Type I growth factor receptor family (EGFR, c-erbB-2, c-erbB-3) is known to be involved in breast cancer development and progression. EGFR is known as an indicator of endocrine independence of breast cancer, c-erbB-2 were first reported in primary breast cancer. Over expression of c-erbB-2 is almost 30% with poor prognosis. Elevated expression of c-erbB-3 has been described, but its association with classical prognostic factors and with clinical outcome is controversial. Expression of MDR1 is widely considered to play an important role in conferring resistance to neo-adjuvant chemotherapy in women with breast cancer. Androgen receptor gene also has a crucial role in the proliferation and progression of breast cancer. AR is detectable in the majority of tumor specimens from patient undergoing mastectomy for breast cancer.

This study had been undertaken to analyze the quantitative expression of type 1 growth factor receptor genes including (EGFR, c-erbB-2, c-erbB-3), AR and MDR1 genes in locally advanced breast cancer and their correlation with response to neo-adjuvant chemotherapy.

Work done during the period

Thirty eight matched samples of pre and post NACT tumor tissues have been collected during the year under report. Total RNA (TRIzol method) was isolated from thirty matched samples 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 (Fig 1). 16 out of 38 were pre-menopausal and 22 were post-menopausal. Relative quantitation expression of EGFR, c-erbB-3 and MDR1 genes were done.

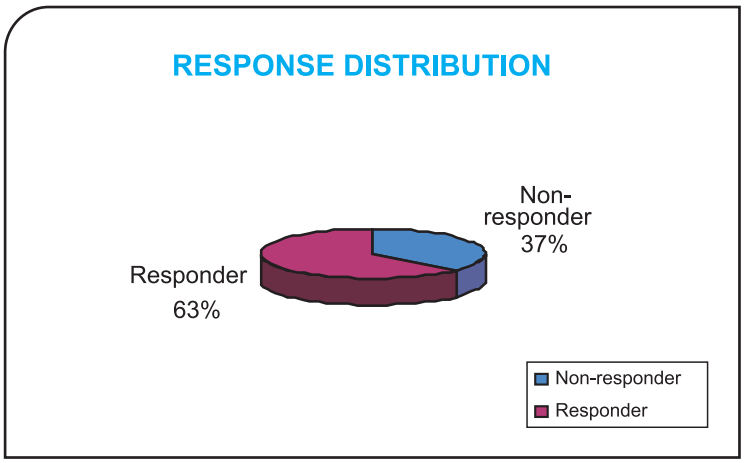


Fig. 1: Shows distribution of patients in terms of response to NACT (n = 38)

Higher expression of EGFR gene was found in post-NACT samples compared to pre-NACT samples (p = 0.034) in non-responder as well as non-responders vs responders (p=0.003) (Fig. 2). Higher expression of c-erbB-3 (p = 0.036) gene was found in post-NACT samples in non-responder group compared to responder group of patients (Fig. 3). Higher expression of c-erbB-3 (p=0.048) was found in post-NACT samples compared to pre-NACT samples in non-responder group of patients (Fig. 3). However, expression of MDR1 (p= 0.017) gene in post-NACT samples in responder group of patients was found significantly high compared to pre-NACT samples (Fig. 4).

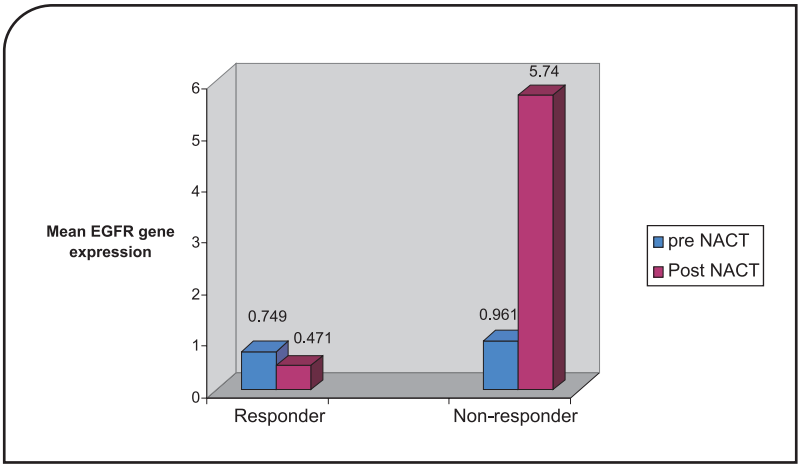


Fig. 2: Shows mean EGFR gene expression changes in response to NACT (Responders vs Non-responders)

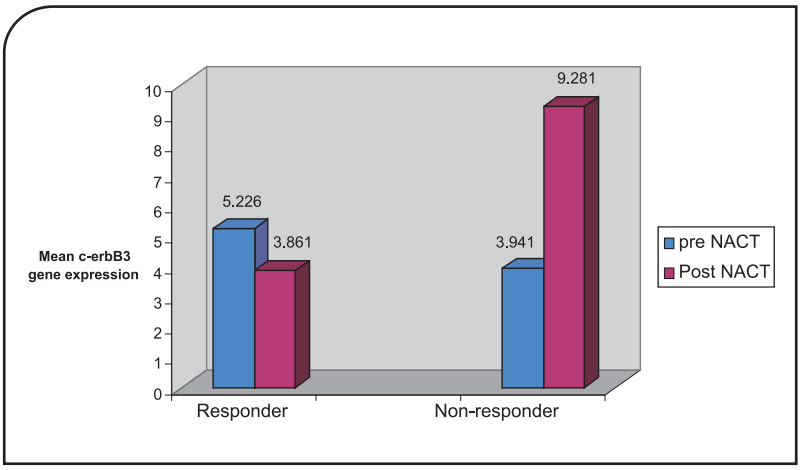


Fig. 3: Shows mean c-erbB3 gene expression changes in response to NACT (Responders vs Non-responders)

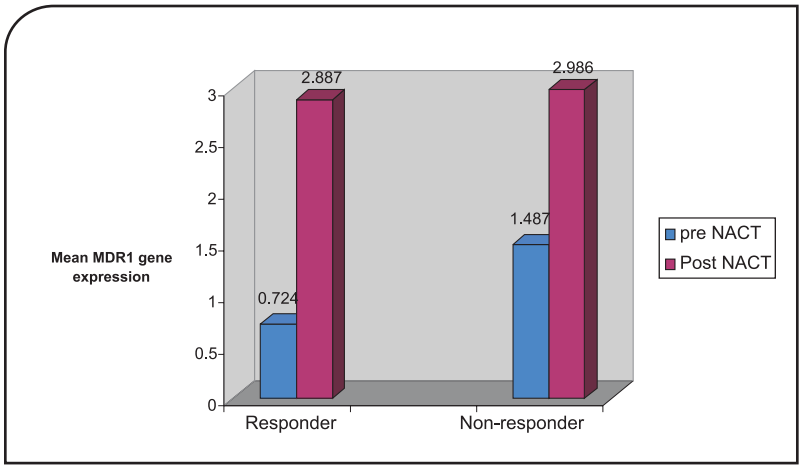


Fig. 4: Shows mean MDR1 gene expression changes in response to NACT (Responders vs Non-responders)

It seems likely that expression of MDR1 gene in breast cancer is associated with a good response to NACT. In non-responders, there is significant increase in levels of EGFR and c-erbB3 in post-NACT samples compared to pre-NACT. No significant difference was found in levels of EGFR, c-erbB3 and MDR1 in pre-NACT samples in responders compared to non-responders. However, non-responders showed significant increased expression of EGFR and c-erbB3 in post-NACT samples.

More number of cases will be included. The role of AR gene expression with other AR pathway genes (TGFB, PSA, ARA70 and SMAD3) & its correlation with EGFR, c-erbB2 and c-erbB3 will be done using Real time PCR.

5. STUDY OF VITAMIN D RECEPTOR POLYMORPHISM AND RETINOID EXPRESSION IN BREAST CARCINOMA

Scientific Staff	:	Dhirendra Yadav, Monika Sharma, Dr. Usha Agrawal, Dr. Sunita Saxena
Collaborators	:	Dr. Dinesh Bhatnagar, Dr. Chintamani Dr. R Mohil, Dr. A Bhatnagar, Safdarjang hospital
Duration	:	2007-2009

Aims, Objectives and Background

Breast cancer is slowly replacing cervical cancer as the leading cancer in females in India. Immunohistochemical expression of hormone receptors (ER, PR) and Her2neu are routinely performed for evaluation of effect of hormonal therapy in patients. Recent interest focuses on the potential effect of Vitamin D and Retinoids. Variations in the Vitamin D receptor gene has been reported and includes single nucleotide polymorphisms at sites cleaved by Bsm1, Apa1, Taq1, Fok1 and poly(A). The Fok1 polymorphism (T/C transition) affects the aminoacid sequence altering the Vitamin D receptor protein structure and creates an upstream initiation codon, resulting in a molecule elongated by three amino acids (f) compared with those initiating translation from downstream site (F). Retinoid acts via two families of nuclear receptors: RARs and RXRs.

The distribution of Vitamin D gene single nucleotide polymorphism with Fok1 restriction enzyme was studied in early onset (<35 years), familial and hormone receptor (ER) positive cases. Data was analysed to look for the prognostic role of these receptors as well as the significance of Fok1 polymorphism in susceptibility to breast cancer.

Work done during the period

The distribution of Vitamin D gene single nucleotide polymorphism with Fok1 restriction enzyme was studied in early onset (<35 years), familial and hormone receptor (ER) positive cases. Blood was obtained from 72 histologically proven breast cancer cases and 70 controls and DNA was extracted by alcohol-chloroform method. The study group constituted 67 (93%) female patients and 5 (7%) male patients. The commonest morphological type was Infiltrating ductal carcinoma (91.7%). The remaining cases constituted 2 of infiltrating lobular carcinoma,

1 each of in situ, medullary, apocrine and tubular carcinoma. Early onset of disease was seen in 21 (29.2%) cases. Family history of cancer in close family members and siblings was present in 11 (16.7%) cases. Estrogen receptor expression was observed in 40 (55.6%) cases. ER+ cases showed both RARA and RXRA concurrently positive (Fig. 1) in 37 cases ($p<0.001$). Parous women had a significantly higher expression of retinoids in comparison to nulliparous women ($p<0.05$).

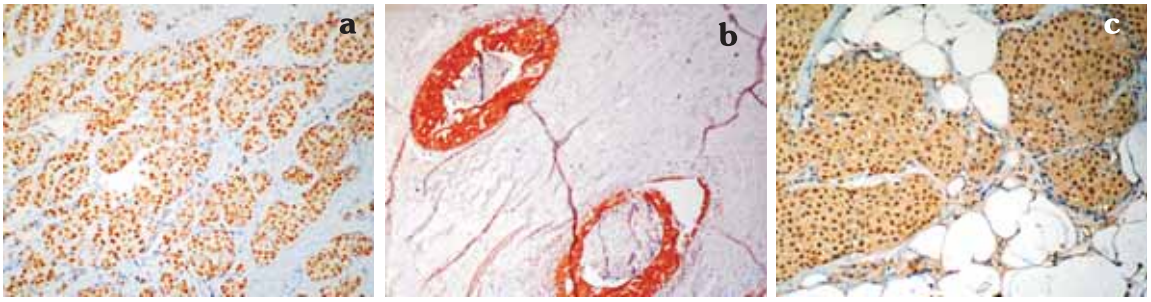


Fig. 1: a. Estrogen receptor expression in nuclei of a case of Infiltrating ductal carcinoma
b. RARA expression in nuclei and cytoplasm of a case of DCIS
c. RXRA expression both in nuclei and cytoplasm of a case of Infiltratng ductal carcinoma

Analysis of Fok1 polymorphism (Fig. 2) in cases showed wild type FF genotype in 33(45.8%) and variant genotype (Ff/ff) in 39(54.2%) cases. The controls showed FF genotype in 60% cases. Both early onset and family history showed no significant association with Fok1 genotype when compared to late onset and sporadic cases. Estrogen receptor (ER) expression was not associated with any particular genotype. Ff and Ff/ff genotypes were associated with significantly increased risk of breast cancer compared to FF genotype ($p=0.045$). RARA/RXRA positive cases constituted 92.5% of the ER positive cases indicating the role of synthetic retinoids in therapy of estrogen positive cases.

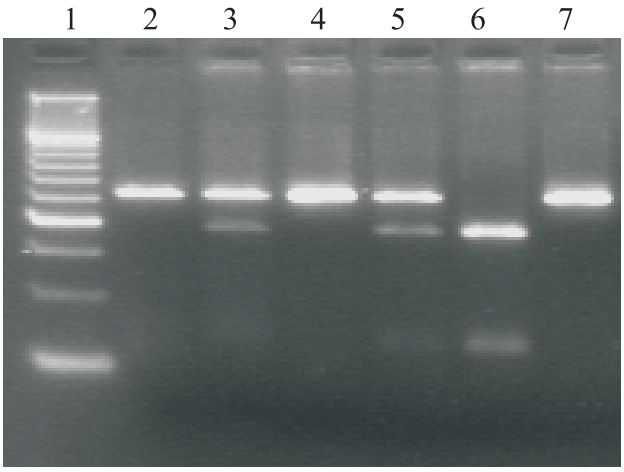


Fig. 2: PCR-RFLP Image of VDR Fok-I polymorphism
Lane 1 : Size Marker (50 bp ladder)
Lane 2, 4 & 7 : Unexcisable fragment- FF (only single band at 265)
Lane 6 : Excisable fragment - ff (Two bands at 169 and 96 bp)
Lane 3 & 5 : Heterozygous - Ff (All three bands at 265, 169 and 96 bp)

B. UROGENITAL MALIGNANCIES

1. STUDY ON MICROSATELLITE INSTABILITY IN ANDROGEN RECEPTOR (AR) AND CYP19, PSA GENE POLYMORPHISM, P53 GENE POLYMORPHISMS AND EXPRESSION PROFILE OF MISMATCH REPAIR GENES IN PROSTATE CARCINOMA

Scientific Staff	:	Dr. Sunita Saxena, Dr. Anju Bansal, Abha Soni
Collaborators	:	Dr. N.K. Mohanty, Safdarjang hospital
Duration	:	2006-2009

Aims, Objectives and Background

Prostate cancer is one of the most prevalent malignancies worldwide affecting the human male population and is predicted to be the third leading cause of cancer deaths in men. Knowing the widespread prevalence of prostate cancer (PCa) in the world and palliative nature of treatment

after the disease spreads, the search for reliable molecular biomarkers is as important as ever. By regulating cell proliferation, differentiation and apoptosis, the androgen receptor (AR) plays a pivotal role in normal prostate development as well as in PCa progression. AR acts as a transcription factor regulating the Prostate-Specific Antigen (PSA) gene by interactions with Androgen-Responsive Element (ARE). One ARE is located in the proximal promoter at -156 to -170 bp from the transcriptional start site of the gene and contains a polymorphic locus (rs266882) at -158 (G to A substitution). Aromatase encoded by *CYP19* is a key enzyme that converts testosterone into estrogen in males, and is suggested to play an important role in the development of benign prostate hyperplasia and prostate cancer. The *CYP19* has a TTTA repeat polymorphism in intron 4 and the polymorphism is reportedly associated with a risk of breast cancer, prostate cancer, and postmenopausal bone metabolism. The p53 tumor suppressor gene (p53) is one of the most commonly mutated genes in all types of human cancer. Defects in mismatch repair (MMR) proteins have been identified in various types of cancer. Defective MMR results in genome instability with detrimental consequences that significantly contribute to tumorigenesis. Therefore, this study had been undertaken to investigate the association of androgen signaling (*AR*, *PSA*) and metabolizing (*CYP19*) genes, p53gene and MMR genes with prostate cancer risk and progression.

Work done during the period

Blood samples from 112 patients of Cancer Prostate (CaP), 126 cases of Benign Prostatic Hyperplasia (BPH) and 106 of healthy normal individuals were collected during the period Nov. 2006 to July 2009. DNA was extracted from all these blood samples using standard phenol-chloroform extraction procedure.

DNA extraction has also been done from formalin-fixed paraffin-embedded tissue samples (FFPE) : Tissue blocks of biopsy samples from 48 CaP and 25 BPH cases. Fresh Tissue samples from 30 cancer prostate cases and 126 BPH cases were also collected in RNA later. RNA has been extracted from 30 cases of CaP and 32 BPH samples and RNA has been converted to cDNA and stored at -30^{o}C.

Gene scan analysis for the identification and sizing of CAG repeats in AR and [TTTA] repeat of CYP19 genes has been done on 105 cases of CaP, 120 BPH and 106 control samples by 3130*xl* Genetic Analyzer. Repeat number was confirmed by sequencing DNA samples harboring alleles of different sizes for both *AR* CAG and *CYP19* TTTA repeats by using the Big Dye Terminator Cycle Sequencing Kit. SNP in the 5'-UTRs of Prostate Specific Antigen

(PSA) gene (G-158A located within PSA ARE1) and codon 72 polymorphism on 4th exon of tumour suppressor p53 gene were also studied by PCR-RFLP assay. Mutational Analysis of core promoter of Mismatch Repair Gene *hMLH1,* by sequencing has been initiated to identify germ-line mutations or polymorphisms. We have identified a C/T transition at position -93 of *hMLH1* which has not yet been reported in CaP. This variation was then genotyped using SNaPshot methodology. Expression profile of Mismatch Repair (MMR) genes, h*MLH1, hMSH2, PMS1, PMS2, MSH3, MSH6:* Real time PCR for MLH1 and MSH2 has been standardized using GAPDH, TBP and 18S RNA as endogenous control in CaP and BPH cases.

Genotypic analysis of *AR* CAG repeats showed a significant association between *AR* CAG repeats and CaP risk. The CAG repeat length of 24 conferred 20% increase in the risk of prostate cancer (OR 2.01 95% C.I., 081-5.03, p=0.01). Also, significance was observed between CaP and BPH with OR of 2.01 95% C.I., 081-5.03, p=0.01. In addition on follow-up, we found 7 androgen resistant cases. In all the cases the CAG repeat length was found to be less than that of mean repeat size. Our results on PSA G>A polymorphism (Fig. 1) showed that the GG genotype is playing a protective role against the risk of prostate cancer with OR 0.26 (95% CI, 0.81- 0.86, p=0.03).

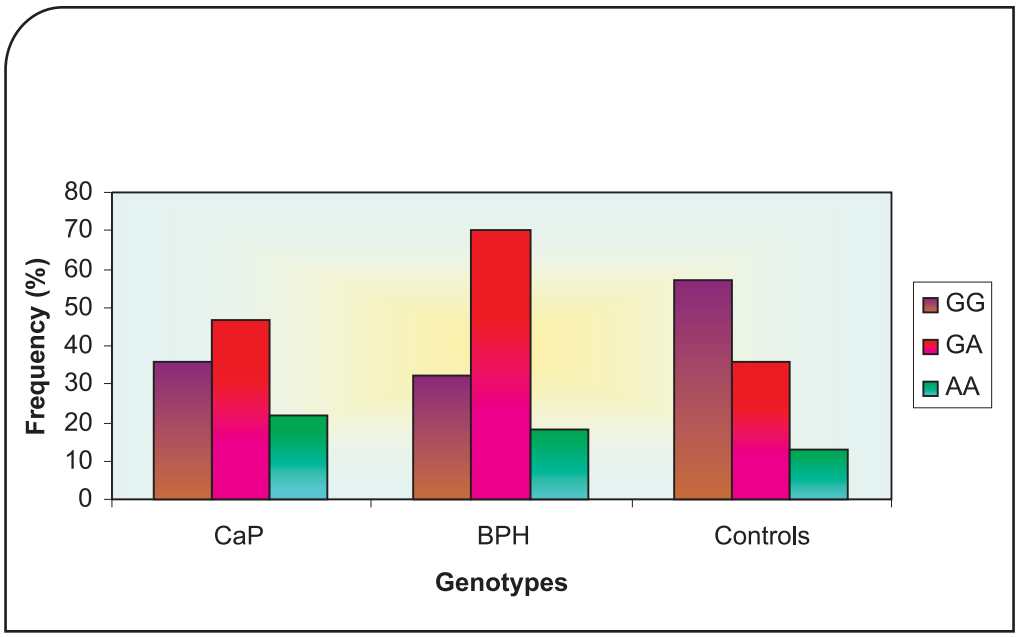


Fig .1: Genotypic distribution of -158 G>A polymorphism in PSA gene

Seven different alleles (designated A1 to A7), of the tetranucleotide (TTTA)n repeat polymorphism of the CYP19 gene were identified. Our results evidenced that homozygosity for the A2 (8 repeats) allele was more common in the CaP than in the BPH or control groups. Heterozygosity for the A1 and A2 alleles (A1A2 genotype) was less common in the CaP (17 [2%]) compared to the BPH and control groups (24 [%]). Risk analysis showed an increased risk of CaP for harboring at least one high risk allele (9 or more TTTA repeats) when all CaP were considered (0R = 1.29, 95% CI 0.83–2.00). There was a 8.6%, 70% and 68% increased risk of prostate caner for men having at least one allele of 9 repeat length ([OR 8.64 95% CI 3.48-21.79, p<0.001], [OR 70.83 95% CI 18.36-281-95, p<0.001], OR 68.67 95% CI 13.25-367.14, p<0.001) respectively for men bearing either heterozygous or homozygous high risk allele. Harboring the homozygote high risk genotypes further significantly increased the risk of carcinoma prostate.

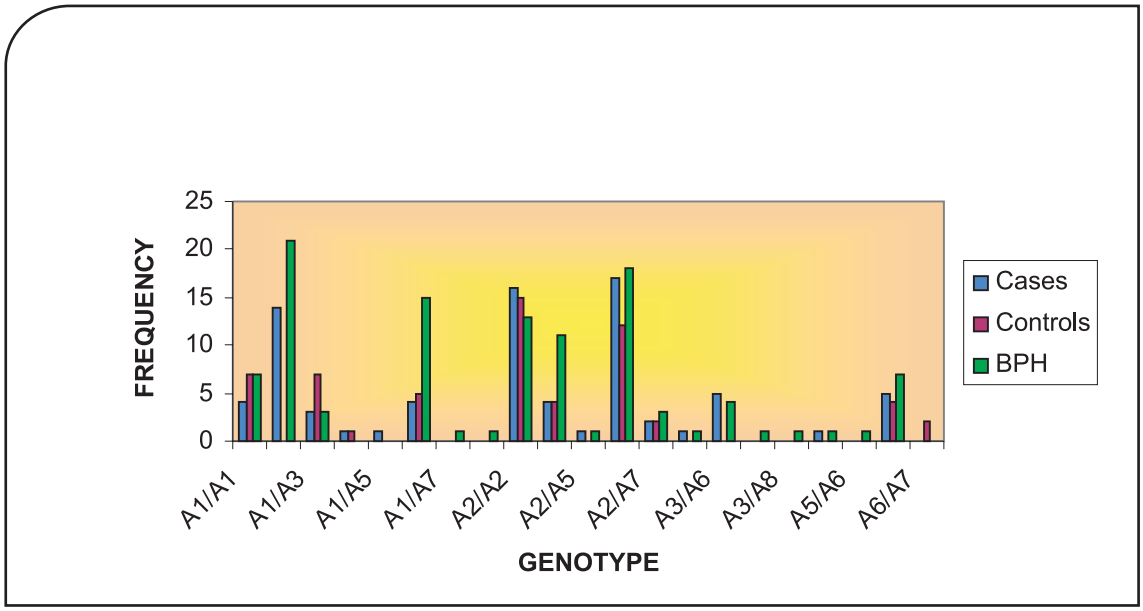


Fig 2: Genotypic distribution of CYP19 [TTTA] repeat polymorphism.

Our results (Fig. 3) on p53 codon 72 polymorphism showed a highly statistically significant association of p53 codon 72 Pro/Pro genotype between patients of CaP and Controls (OR= 4.322, 95% CI, 1.91-9.74, $p \leq 0.001$), as well as between BPH and Controls (OR= 4.45, 95% CI, 1.96- 10.08, $p \leq 0.003$). However genotypic association between CaP and BPH ($p=0.167$) was not found significant. These findings are consistent with impaired DNA repair associated with the 72Pro p53 protein.

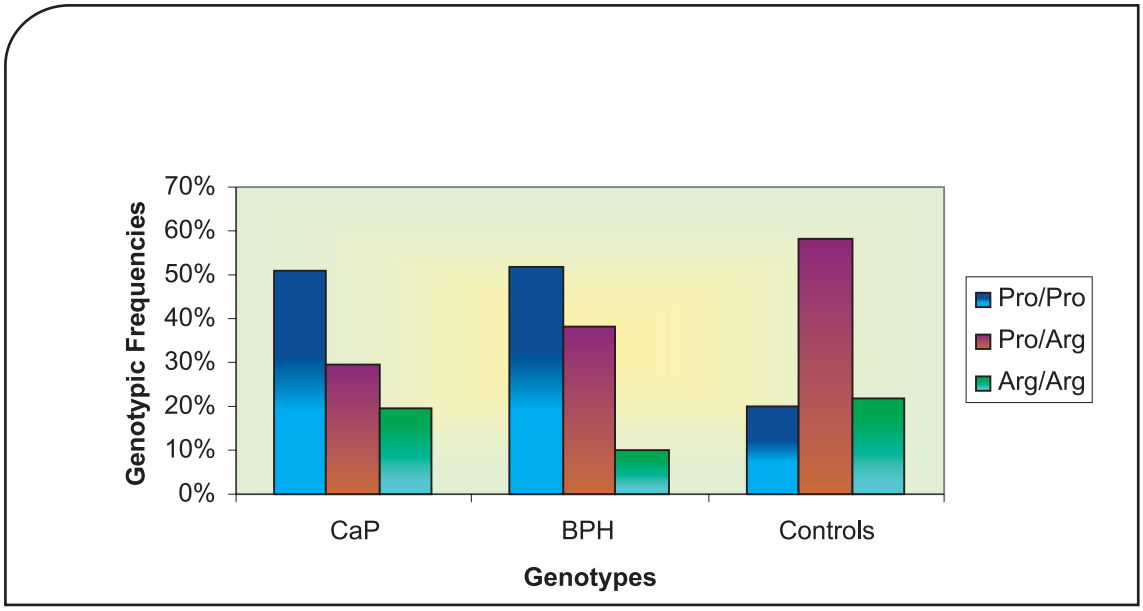


Fig. 3: Genotypic distribution of p53 codon 72 polymorphism

A C/T transition at -93 position of the core promoter region of MLH1 gene was also identified and evaluated using SNaPshot genotyping methodology. We investigated and found that CC genotype increases the risk of prostate carcinoma as the differences in the distribution of -93 C/T polymorphism between the CaP cases and controls, BPH and Controls were statistically significant with OR= 1.961, 95% CI, 1.02-3.76, p= 0.05, OR= 2.04, 95% CI, 1.08-3.86, p= 0.039 respectively. The findings of the present study indicate that TT genotype may represent a genetic predisposing factor for prostate cancer development (Fig. 4). For the analysis of MMR gene expression, RNA has been isolated from 30 cases of CaP and 32 cases of BPH as controls. Real time PCR has been standarised using three endogenous controls *viz.* GAPDH, TBP and 18S RNA genes. Further we are analyzing cases and control samples.

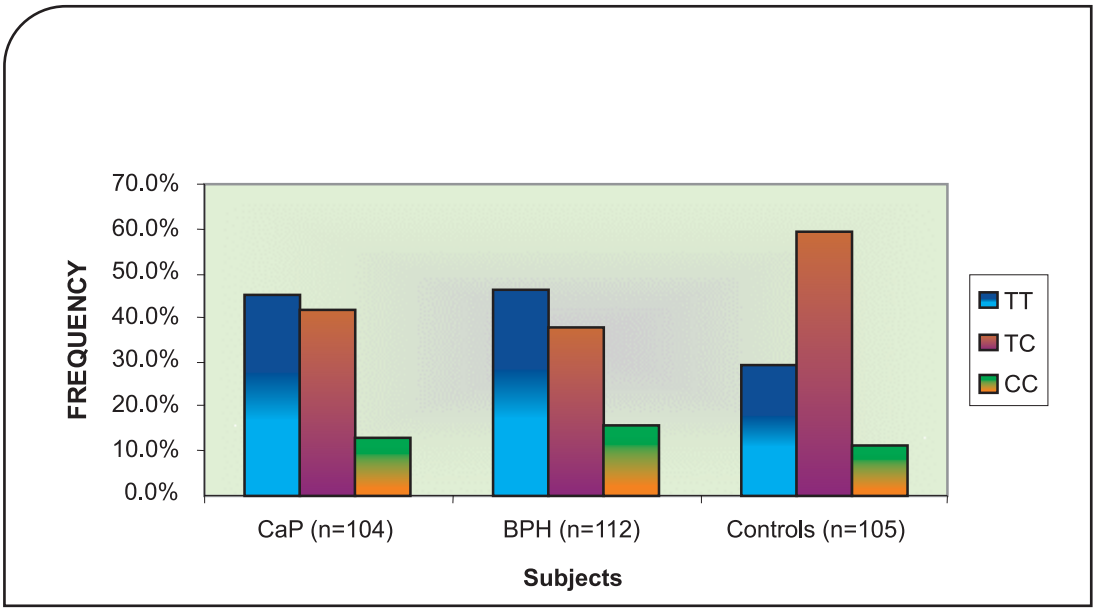


Fig. 4: Genotypic distribution of -93 C/T transition in MLH1 gene

2. TO STUDY THE ROLE OF CYCLOOXYGENASES IN CYTOKINES DYSFUNCTION OF INVASIVE AND NON-INVASIVE TRANSITIONAL CELL CARCINOMA OF HUMAN BLADDER

Scientific Staff	:	Dr. Saurabh Varma, Dr. Usha Agrawal, Dr. Sunita Saxena
Collaborators	:	Dr. N. K. Mohanty, Safdarjang hospital
Technical Staff	:	Mr. P. D. Sharma
Duration	:	2007-2010

Aims, Objectives and Background

Cyclooxygenases catalyzes the formation of prostaglandins from arachidonic acid, is up-regulated in multiple types of solid tumors, including urinary bladder in humans. Prostaglandin has been a major cox product involved in tumor development and progression. Cyclooxygenase derived prostaglandins contribute to tumor cell resistance to apoptosis, new blood vessel formation, and tumor cell proliferation.

In this study, we have evaluated the role of cyclooxygenases and associated cytokines in PBMCs and tumour infiltrating lymphocytes to understand the role of cyclooxygenase in modulating the PBMCs cytokines and their effector function in invasive and non-invasive TCC of human bladder.

Work done during the period

Heparinized blood samples from 10 normal healthy individuals and 46 TCC patients from Urology Department, Safdarjung Hospital were collected for the study of Cox-1 and Cox-2 expression in correlation with cytokines study by Flow Cytometer. Tissue from tumour 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 invasive and 40 were non-invasive. Out of six invasive cases, 2 and 4 were from grade II and grade III respectively while out of 46 non-invasive cases, 6, 29 and 5 were from grade I, II and III respectively.

In case of non tumrous bladder urothelium taken from patients, Cox-2 expression was absent. In tumour tissue, Cox-2 expression was not seen in low grade (Grade I) tumors in non-invasive cases. Grade II and III tumours of invasive category showed 50 and 100% expression

respectively while in non-invasive category, Cox-2 expression was 60% and 76% in grade II and grade III tumours respectively.

Cox-1 expression was observed in 70% cases in normal epithelium using both flurorescent and IHC techniques. Its expression in non-invasive cases was 83, 84 and 77% in grades I, II and III respectively. While in invasive group, Cox-1 expression was about 50% in both grade II and III tumours.

The Flow Cytometric studies on PBMCs from normal healthy individuals showed the maximum expression of IL-1β. Cox-2 expression was found increased in cancer patients in comparison to normal healthy individuals The mean percentage of double positive cells of IL-1β and TNF along 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 seen in 4 out of 5 invasive TCC samples. Significant increase in IL-1Beta ($p>0.004$) and IL-6 ($p>0.01$) level was observed in cancer patients in comparison to normal group. The higher expression of CD74($p>0.002$) was found in cancerous patients compared to control group.

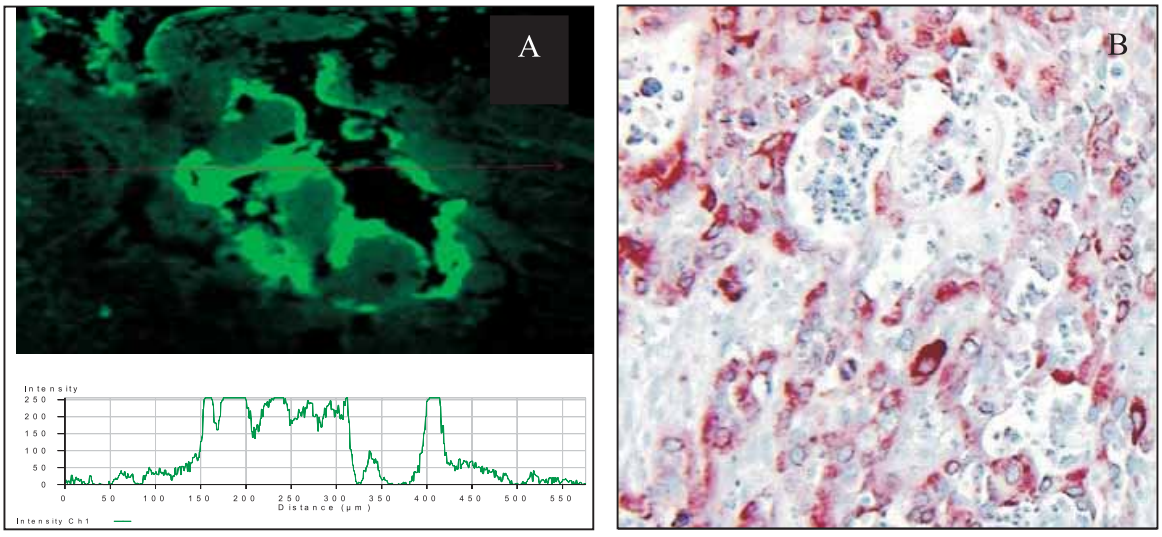


Fig 1 A and B : Showing Cox-2 expression using CLSM and IHC in invasive TCC.

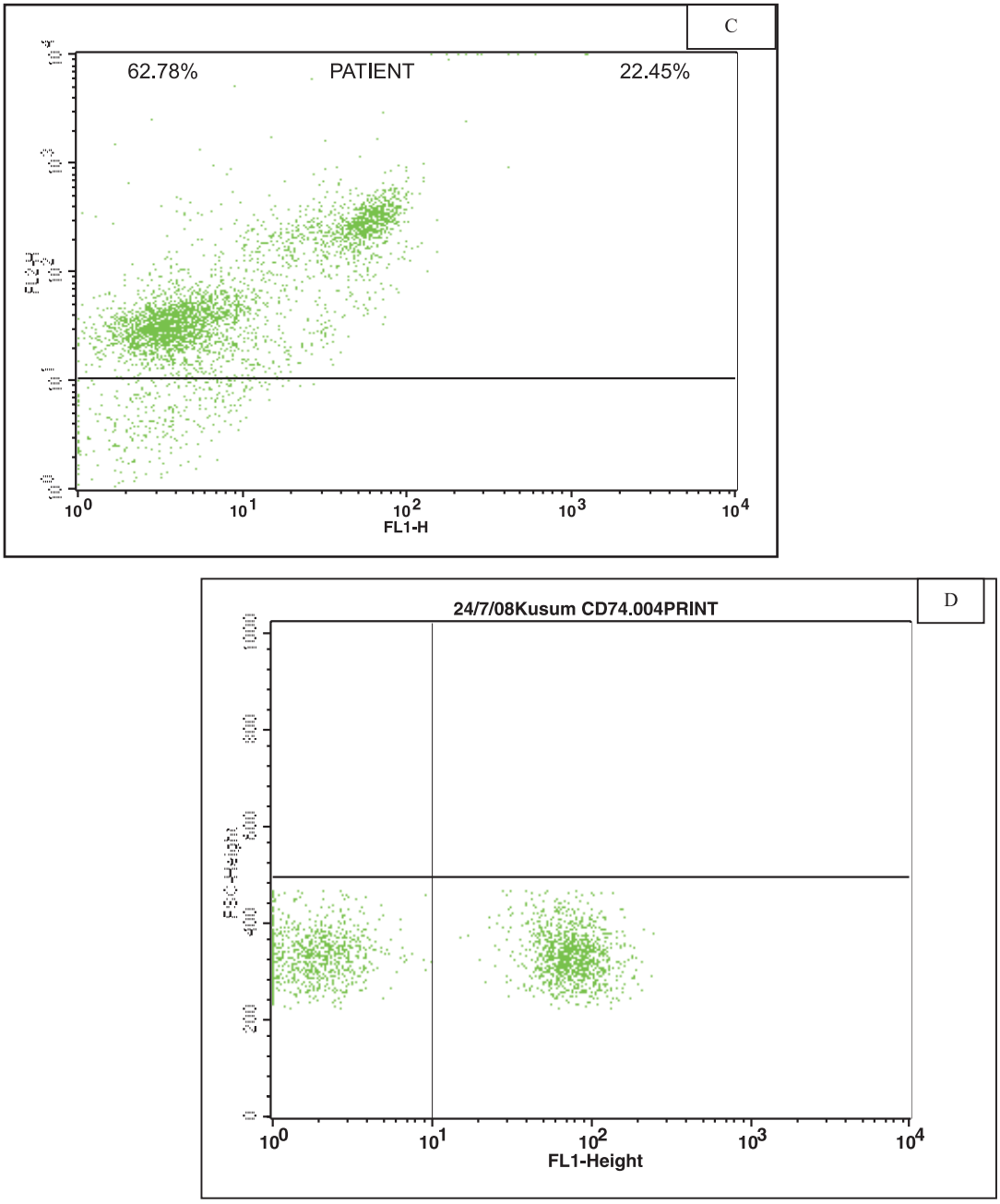


Fig. 2 C and D : Showing expression of Cox-2/IL-6 and CD74 using Flow Cytometry.

C. CANCER IN NORTH-EAST REGION OF INDIA

NE Indians have diverse ethnic groups, customs, food habits and life style. A high incidence of several cancers such as those of esophagus, oral, lung, stomach, breast and NHL have been reported from these regions. In addition, familial aggregation of esophageal cancer has been reported in Assam. These cancers are often associated with exposure to tobacco and pesticides. The form in which tobacco is consumed is different in NE region. Moreover, there is extensive use of pesticides in tea gardens in these regions. A Multi-centric study was initiated by ICMR to find out if genetic factors, in addition to environmental exposure could possibly explain the high prevalence and familial aggregation of esophageal cancers. The study was later extended to other tobacco related cancers including oral, gastric and lung cancers and two pesticide related cancers including breast cancer and lymphoma. These projects are in collaboration with Dr. Bhubaneshwar Borooah Cancer Institute, Guwahati, Assam, Regional Medical Research Centre, ICMR, Dibrugarh, Assam, Institute of Cytology and Preventive Oncology, ICMR, Noida, U.P., National Institute of Occupational Health, Ahmedabad, National Cancer Registry Programme, Bangalore, and the Population Based Cancer Registers (PBCRs) of NE India.

1. COMPREHENSIVE STUDY OF CARCINOMA OESOPHAGUS AT NORTHEAST INDIA - MULTIDISCIPLINE APPROACH

Scientific Staff	:	Dr Sunita Saxena, Dr. Sujala Kapur, Indranil Chatterjee
Collaborators	:	Dr. J. Mahanta, RMRC. Dibrugarh, Assam Dr. A. C. Kataki, BBCI, Guwahati, Assam Dr. B. C. Das, ICPO, Noida
Duration	:	2004-2009

Aims, Objectives and Background

A high prevalence of oesophageal cancer with familial clustering has been reported from certain regions of NE India. The cause for this is not known. The current study aims to analyze differential gene expression patterns and frequency of allelic loss or loss of heterozygosity in microsatellite markers in familial and non-familial esophageal cancer patients and also with

respect to tobacco chewing and non-tobacco chewing habit. For gene expression profile studies, tumor and matched normal tissue from esophageal squamous cell carcinoma patients with a family history of upper gastrointestinal cancer were analyzed using cDNA microarray chips containing 10,000 genes.

Work done during the period

The gene expression profile of esophageal cancer (EC) in high-risk area of India (Assam) was studied using cDNA microarray. Nine biopsy samples from esophageal squamous cell carcinoma cases with a family history of upper gastrointestinal cancer were compared with normal pooled esophageal tissue. Using stringent criteria ($p \leq 0.05$ and ≥ 1.2 fold change) and Gene Ontology, 350 differentially expressed genes (26 upregulated and 324 downregulated) were identified in tumor tissue and categorized into known or probable functional categories on the basis of biological processes and molecular function. Genes involved in cell motility, anti-apoptosis, glucocorticoid receptor activity, steroid hormone receptor activity, arginase activity and metabotropic glutamate, GABA-B-like receptor activity were significantly upregulated. Genes involved in extracellular matrix organization, BMP signaling pathway, glutamate receptor activity, epoxide hydrolase activity, apoptogenic cytochrome c release channel activity, ion transport, response to stress and MAP kinase activity were significantly downregulated. Validation of microarray results with quantitative real time PCR for five genes was done confirming that the genes identified in microarray analysis have significant and progressive changes in gene expression.

Validation of the cytokeratin profile at the protein level in EC has been done by tissue microarray. 120 TMA cores with core cylinder diameter of 1.5 mm corresponding with high risk, low risk and normal controls on the manual BEECHER Tissue Arrayer were constructed. Immunohistochemical expression of CK 4,5,8,14,17 was done. The data was analyzed using SPSS software. Cytokeratin (CK) 4 was downregulated while CK 5,8,14,17 were overexpressed at the protein level. CK4, 5,17 expression was significantly different between high risk and low risk tumors. CK8, 17 were statistically significant in distinguishing normal squamous epithelium from the tumor in high-risk cancers. CK14 is not a good marker to define normal epithelium from the neoplastic epithelium by IHC. Analysis by ANOVA did not show any statistical difference in expression of Ck14 between normal esophageal epithelium and different grades of squamous cell carcinoma.

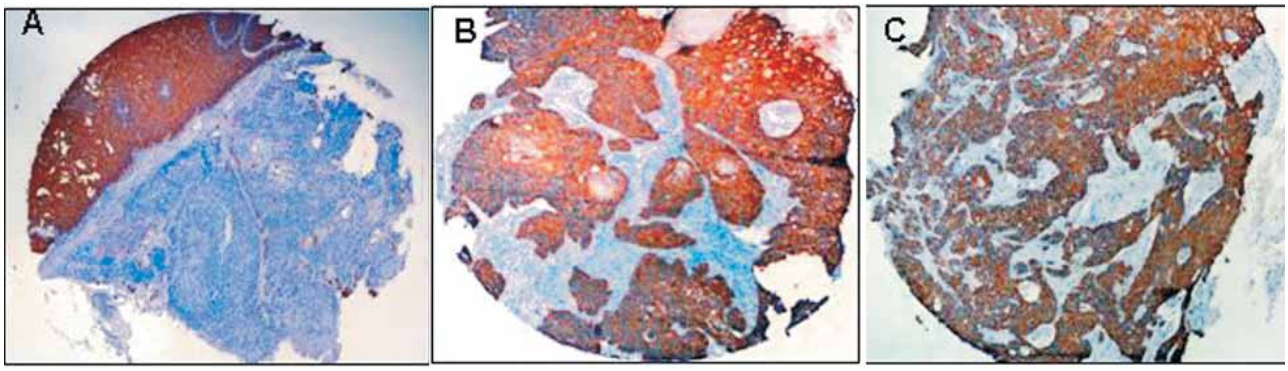


Fig. 1: TMA cores of ESCC showing (A) no immunoexpression of CK4 and positive immunolabelling with CK8 (B) and CK14(C)

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 were 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.2).

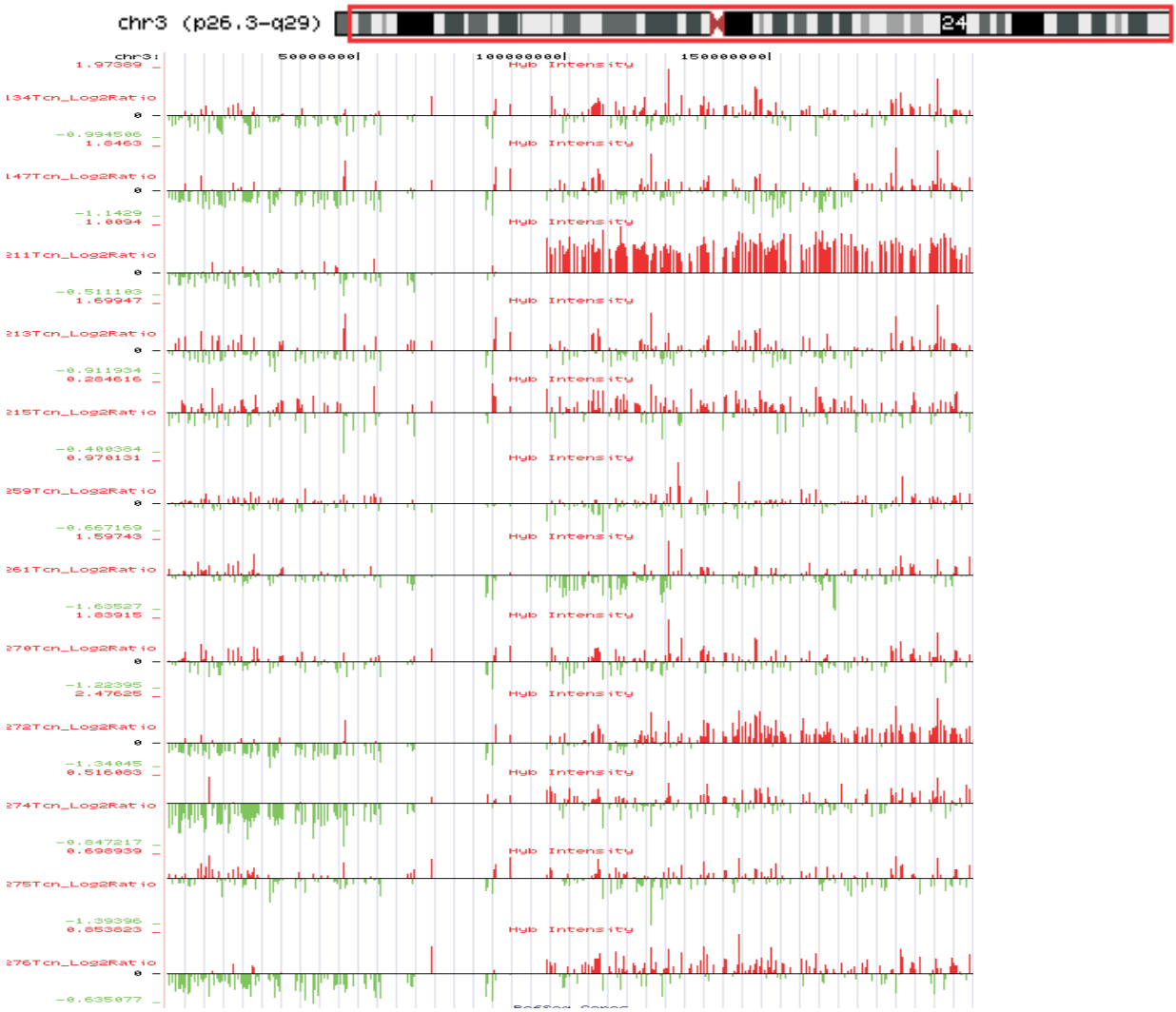


Fig. 2: Copy number alteration profile of Chromosome 3 in all tumor samples

2. ROLE OF TOBACCO USE IN CAUSATION OF CANCER IN NORTHEAST INDIA

Scientific Staff	:	Dr Sunita Saxena, Dr. Sujala Kapur, Dr. Usha Agrawal, Th. Regina Devi, Dhirendra Singh Yadav, Rakshan Ihsan
Collaborators	:	Dr. J. Mahanta, RMRC, Dibrugarh, Dr. H.N. Sayed, Dr. P. Nag, NIOH, Ahemedabad, Dr. B.C. Das, Dr Alok Bharti, ICPO, Noida, Dr. A. Nand Kumar, NCRP, Bangalore, Dr. Eric Zomwia, Aizawl, Dr. Yogesh Varma, Sikkim
Duration	:	2005-2010

Aims, Objectives and Background

The carcinogenic potential of tobacco depends on its carcinogenic contents and also on its pattern of use. The form in which tobacco is consumed in NE region is different from that of the rest of the country. A Multicentric study has been designed with NCRP, Bangalore (to design and prepare questionnaire to relate epidemiological data), RMRC, Dibrugarh (for statistical analysis of data and its interaction with other factors to evaluate the risk of cancer) and with NIOH, Ahmedabad (for estimation of metabolites of nicotine and pesticides in serum and urine samples). The role of IOP, New Delhi and ICPO, Noida is to identify risk of developing cancers with relation to allelic alteration in microsatellite markers, mutation/ polymorphisms and gene expression pattern. Blood and tissue samples are being sent to IOP from three PBCR Centers at Dr. B. Boroowah Cancer Hospital, Guwahati, Civil Hospital, Aizawl and Sir TNM Hospital, Gangtok for oral, esophageal, gastric and lung cancers.

Work done during the period

Using statistical analysis, the number of samples that are to be collected from the northeast centers to reach a statistically significant conclusion in this project are 250 for oral cancer, 200 for stomach cancer, 400 for lung cancer and 350 for esophageal cancer with an equal number of age and sex matched controls. Three of the PBCRs (Aizawl, Gangtok and Guwahati) send the tissue and blood samples to IOP and the other three (Silchar, Dibrugarh and Imphal) to ICPO.

Table 1: Tissue and blood samples received from patients and controls

Type of Cancers	Cases		Controls	
	Committed	Tissue & Blood Received	Committed	Received
Esophageal Cancer	175	55 185	175	499 (206 Females)
Oral Cancer	125	88 223	125	
Stomach Cancer	100	122 134	100	
Lung Cancer	200	4 110	200	
Total Tissue Total Blood	600	269 587	600	499

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.

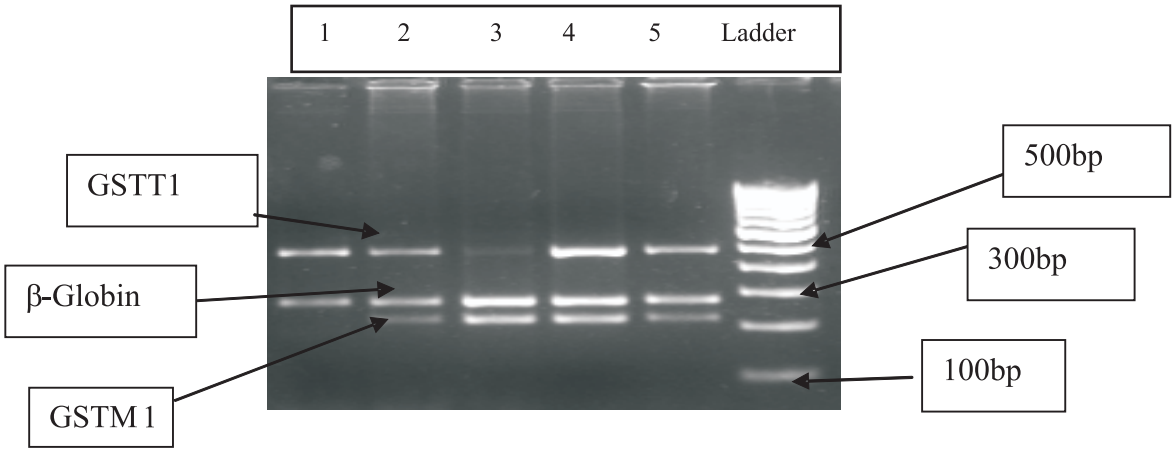


Fig.1: Detection of GSTT1 and GSTM1 in 2% agarose gel electrophoresis. Lane 1 showing M1 null genotype.

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.

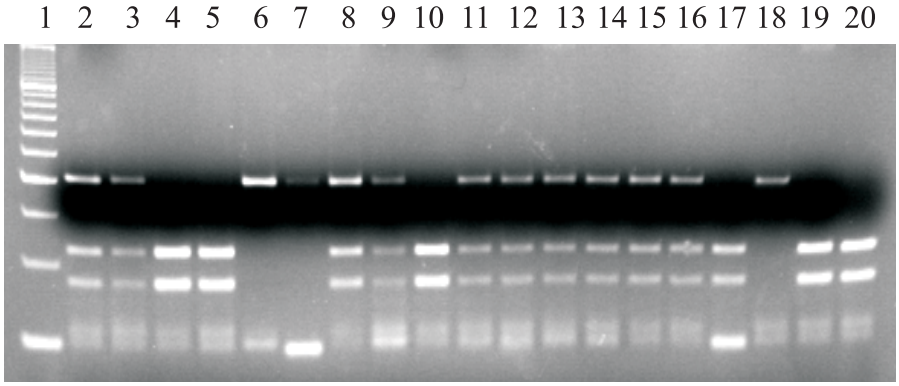


Fig. 2: Analysis of RFLP digested PCR products of codon 72 of p53 gene by agarose gel electrophoresis (3.5%).
Lane 1, 50bp ladder.
Lane 2,3,8,9,11,12,13,14,15 and 16, Arg/Pro (heterozygous)
Lane 4,5,10,17,19 and 20, Arg/Arg (homozygous)allele,
Lane 6,7 and 18, Pro/Pro (homozygous) allele.

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.

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. 3).

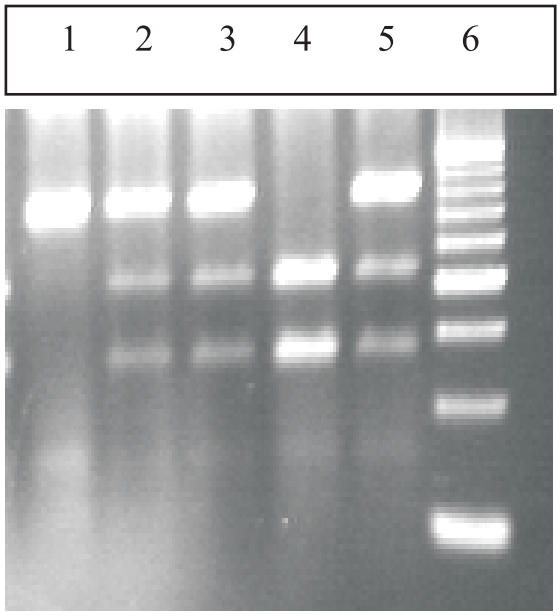


Fig. 3: RFLP digested fragments for Cyp1A1, polymorphism
Wild type (W/W)- single band at 340bp eg.
Lane 1 Homozygous variant allele (V/V)- two bands at 200 and 140 bp eg.
Lane4 Heterozygous Variant allele (W/V) – all three bands present eg. Lane 2,3,5
Lane 6, 50bp ladder

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. 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
- 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 p53 mutations by Single-Strand Conformation Polymorphisms (SSCP) gel electrophoresis

Non-radioactive silver-stained SSCP is a sensitive, rapid, and simple technique to detect p53 mutations, and could be easily used to investigate large series of patients to assess the clinical significance of p53 mutations in human tumors. Exon 6 of p53 gene was amplified by polymerase chain reaction and screening of point mutation was performed by SSCP in 10 cases and 4 normal tissue DNA by SSCP (Fig.4). Sample with a shift in a band pattern was sequenced.

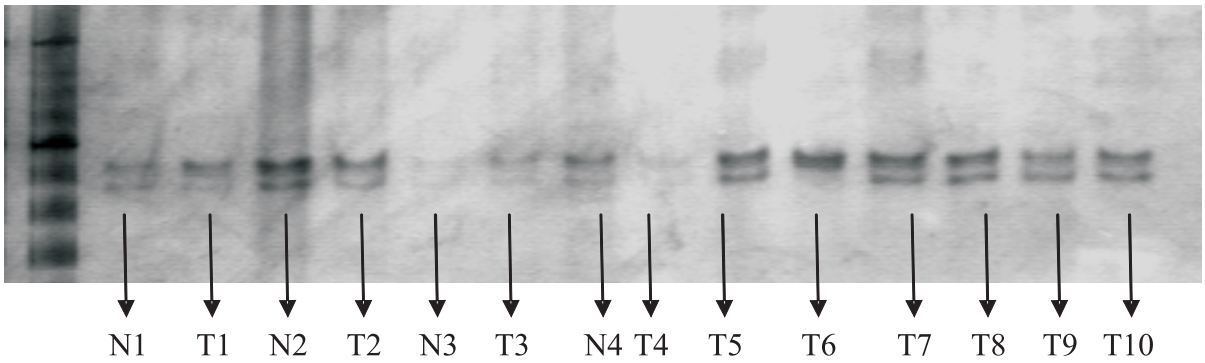


Fig. 4: PCR/SSCP analysis of exon 6 of the p53 gene. Mobility observed in tumor tissue DNA(T) do not differ from that in normal tissue DNA(N).

DNA Sequencing

After SSCP, the DNA for two tumour samples were reamplified using the same primers. The PCR amplified DNAs were purified using QIAquick PCR purification Kit (*Qiagen, Germany*) and the purified product was sequenced on an ABI PRISM 3310 XL automatic sequencer using the dideoxy dye termination method (Fig.5)

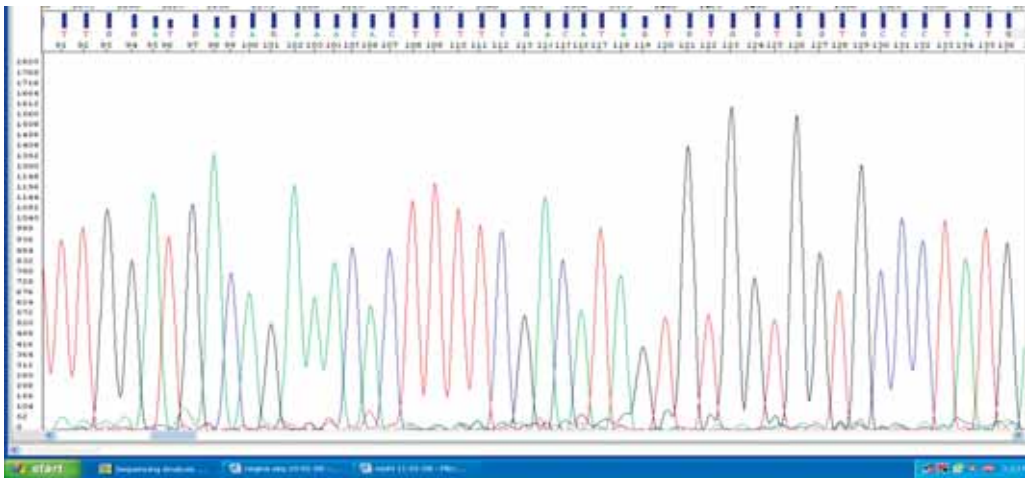


Fig. 5: Electrongram showing the DNA sequence of Exon 6 of p53 in a tumor sample (GW-ST/233-07/TR). No mutation was detected.

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 (Fig. 6) using published primer sequences.

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'

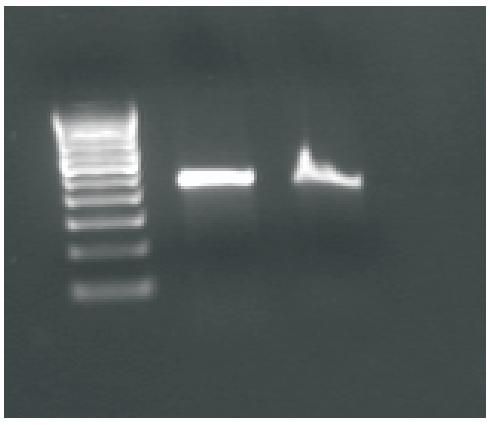


Fig. 6: 1.5 % Gel showing the PCR product of ure A gene. Lane 1- 100bp ladder, Lane 2 & 3 PCR product of 411 bp size

Gene Expression study

High throughput oligonucleotide microarrays from M/s Ocimum Biosolutions were used to compare molecular features of stomach cancers of three samples. Genes involved in biological processes such as cell-cell adhesion, cell motility, DNA synthesis during DNA repair, Ras protein signal transduction and apoptosis were found to be downregulated and genes involved in biological processes such as cell proliferation, regulation of mitosis, endothelial cell migration, inflammatory response and cell-cell signaling were found to be upregulated.

LUNG CANCER

Genotyping of GSTT1 and GSTM1 Genes

89 cases and 21 controls have been studied for GST polymorphism. Of the 89 cases, 29 samples showed null polymorphism for GSTM1, 13 showed null polymorphism for GSTT1 and 2 samples showed both GSTM1/GSTT1 null polymorphism. Of 21 control samples, 2 samples showed null polymorphism for GSTM1, 5 samples showed null polymorphism for GSTT1 and 1 for both GSTM1/GSTT1 null polymorphisms.

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.

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 was detected by PCR-RFLP method by using EcoRV and RsaI restriction enzymes for exon3 and exon 4 respectively (Figs. 7-8).

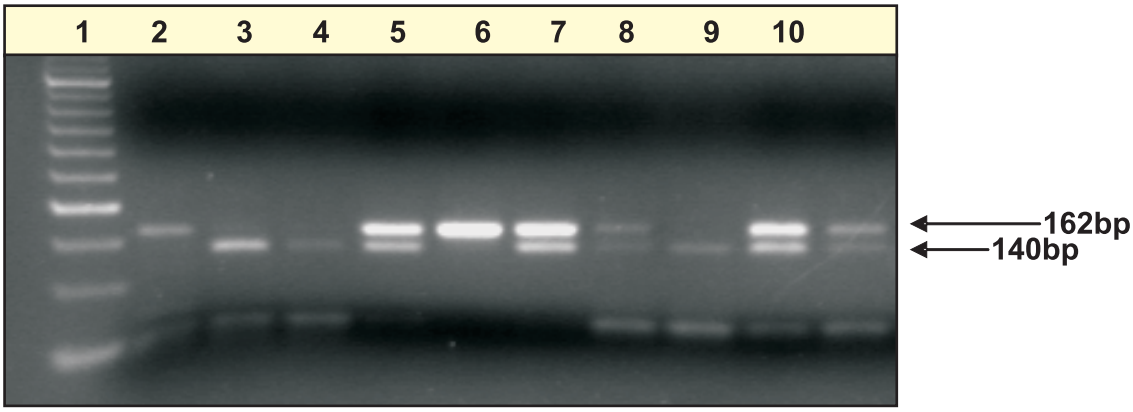


Fig. 7: PCR-RFLP for Exon 3

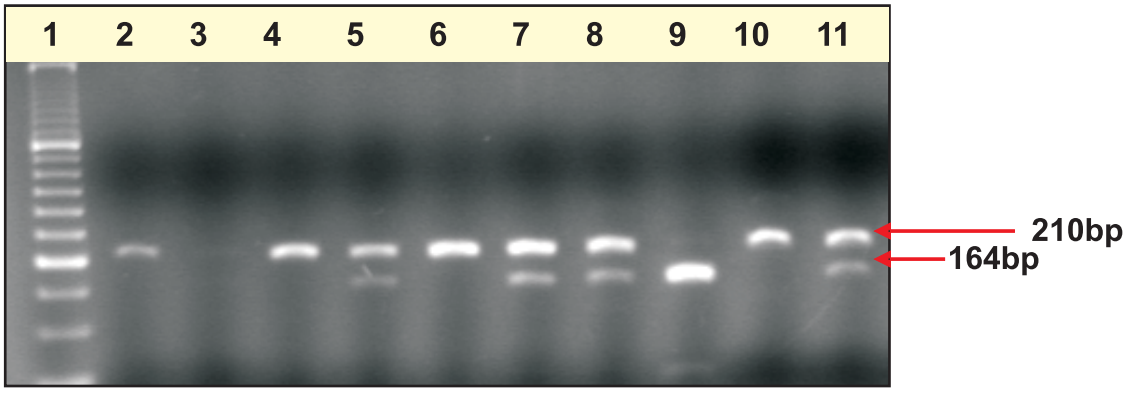


Fig. 8: PCR-RFLP for Exon 4

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.

3. EFFECT OF PESTICIDE EXPOSURE IN CAUSATION OF CANCER IN NORTH EAST INDIA

Scientific Staff	:	Dr Sunita Saxena, Dr. Sujala Kapur, Dr. Usha Agrawal, Mishi Kaushal, Pradeep Singh Chauhan
Collaborators	:	Dr. J. Mahanta, RMRC, Dibrugarh, Dr. H.N. Sayed, Dr. P. Nag, NIOH, Ahemedabad, Dr. B.C. Das, Dr Alok Bharti, ICPO, Noida, Dr. A. Nand Kumar, NCRP, Bangalore, Dr. Eric Zomwia, Aizawl, Dr. Yogesh Varma, Sikkim
Duration	:	2005-2010

Aims, Objectives and Background

There is widespread exposure to pesticides in the tea gardens of the NE India. It is unknown if the high prevalence of certain cancers in NE India could be due to these environmental carcinogens. The current multicentric study has been designed to find out possibility of gene-environment interaction in the occurrence of breast cancer and lymphoma in NE India. Using statistical analysis, the number of samples that were to be collected from the northeast centers to reach a statistically significant conclusion in this project are 150 for breast cancer and 100 for lymphomas with an equal number of age and sex matched controls. Three of the PBCRs (Aizawl, Gangtok and Guwahati) send the tissue and blood samples to IOP and the other three (Silchar, Dibrugarh and Imphal) to ICPO.

Table 1: Tissue and blood samples received from patients and controls

Type of Cancers	Cases		Controls	
	Committed	Received	Committed	Received
Breast Cancer	125	117	125	499 (206 Females)
Non-Hodgkin's Lymphoma	100	84	100	
Total	225	201	225	206

BREAST CANCER

Breast cancer is one of the leading causes of death among Indian women. The differences in breast cancer incidence reflect the diversity of ethnic and religious groups, and have been thus far attributed primarily to variation in social and cultural factors and environmental exposures, with little information available on the possible role of genetic factors. The North Eastern region in India has diverse ethnic populations with different customs, food habits and life style. Northeastern states in India have reported a very high incidence of cancer of all anatomical sites together (ICMR 2004) as well as high incidence of sites of cancer associated with use of tobacco.

Along with the high use of pesticides, the pattern of tobacco usage is different in northeast region where tobacco pastes (gadakhu and gul) and tobacco water (tuibur or hidakphu, manufactured by passing tobacco smoke through water used for gargling) are used in Manipur, Mizoram, Sikkim and Tripura. This region has a different genetic makeup due to ethnicity, environmental conditions and life style than the rest of the country. The architectural features that result in the instability of the genomic regions due to either difference in ethnic background, environmental, life style and/or tobacco usage need to be determined. The specific objectives are:

1. To screen and characterize putative high (BRCA1 BRCA2 and TP53) and low (CYP17, GSTT1, GSTM1, GSTP1, CYP17) susceptibility genes for mutations and polymorphisms.

2. To screen and characterize novel gene copy number alteration and allelic imbalances using high throughput techniques.

CYP17 Polymorphism

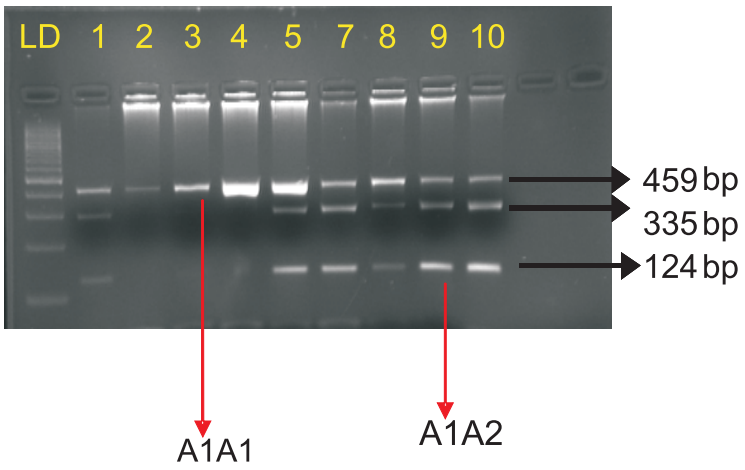


Fig. 1: 3% Agarose Gel showing MspA1 polymorphism by RFLP analysis. Lanes: LD-Ladder, 3-A1A1 (459bp product), 9-A1A2 (459bp, 335bp and 124bp product)

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:

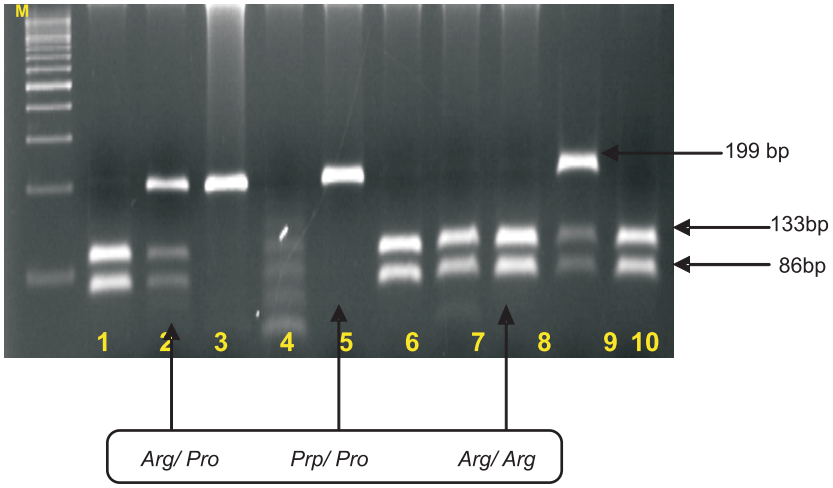


Fig. 2: 3% Agarose gel electrophoresis of Digested product of p53 genes. 133 bp and 86 bp fragment: Arg/ Arg ; 199 bp, 133 bp and 89 bp fragment: Arg/ Pro 199 fragment: Prp/ Pro Lane M: maker;Lanes 2 and 9 : Arg/ Pro; Lanes 3 and 5: Prp/ Pro;Lanes 1,6,7,8,10 : Arg/ Arg

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.

Lymphoma

Environmental or occupational exposures to carcinogens are suspected to be risk factors for lymphoma. Lymphoma is more frequently observed in agricultural as compared to rural populations. Association between NHL and pesticide exposure has been observed repeatedly. A genetic hallmark of non-Hodgkin's lymphoma is the presence of recurrent chromosomal translocation involving the immunoglobulin heavy chain gene. The t (14; 18) occurs in 85-90% of follicular lymphoma and 25%of diffuse B-cell lymphomas. The BCL2-IGH translocation measurements could be a measure of acquired genetic instability in relation to genotoxic exposure in a gene directly relevant in term of lymphomagenesis.

The interplay between genetic susceptibility and exposure to carcinogens in the etiology of lymphoma, may involve the *GSTM1* and *GSTT1* gene polymorphisms that show broad differences in incidence among ethnic groups. Polymorphisms within the *GST* genes may be associated with susceptibility to lymphoma. In the current study, the frequency of polymorphisms resulting in deletions of two genes involved in the detoxification of potentially carcinogenic agents, glutathione *S*-transferase *GSTM1* and *GSTT1,* were analysed in patients with lymphoma. The specific objectives are:

1. To identify commonly occurring translocation for evaluating gentoxicity of pesticide exposure.

2. To study the genetic polymorphism of GST genes in lymphoma cases.

Work done during the period

For Bcl2/IgH tranlation analysis, PBMC cells from a case of follicular lymphoma were lysed with TRIZOL reagent (Invitrogen) and DNA and RNA was recovered. RNA integrity was checked on a 1.2% formaldehyde-agarose gel. cDNA was generated by reverse transcription of total RNA using high capacity cDNA archive kit (Applied Biosystems). RT-PCR was done using cDNA and specific primers of bcl-2/IgH (Fig. 5). Sequencing was done to confirm the presence of the bcl2/IgH translocation.

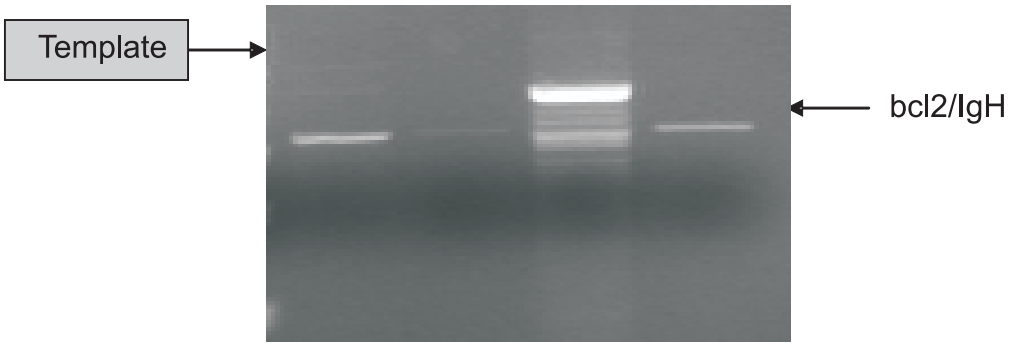


Fig. 3: 2% EtBr stained Agarose gel showing the standardization of RT-PCR for bcl2/IgH in the peripheral blood of the follicular lymphoma patient on DNA and cDNA.

Forty blood samples have been analyzed so for. None of them were positive for the Bcl-2/IgH translocation.

GST Polymorphism

For GST Polymorphism, peripheral blood samples were obtained from both cases and controls and DNA was extracted using SDS–proteinase K and phenol–chloroform method. The GSTM 1 and GSTT1 genetic polymorphisms were evaluated using multiplex polymerase chain reaction (PCR) techniques. The genes were amplified with specific primers and β-globulin

was used as an internal standard. DNA fragments of expected size that is 473 bp and 210bp were amplified using primers specific for GSTT1 and GSTM1 genes, respectively. For internal control, a fragment of size 267 bp was obtained (Fig. 4.).

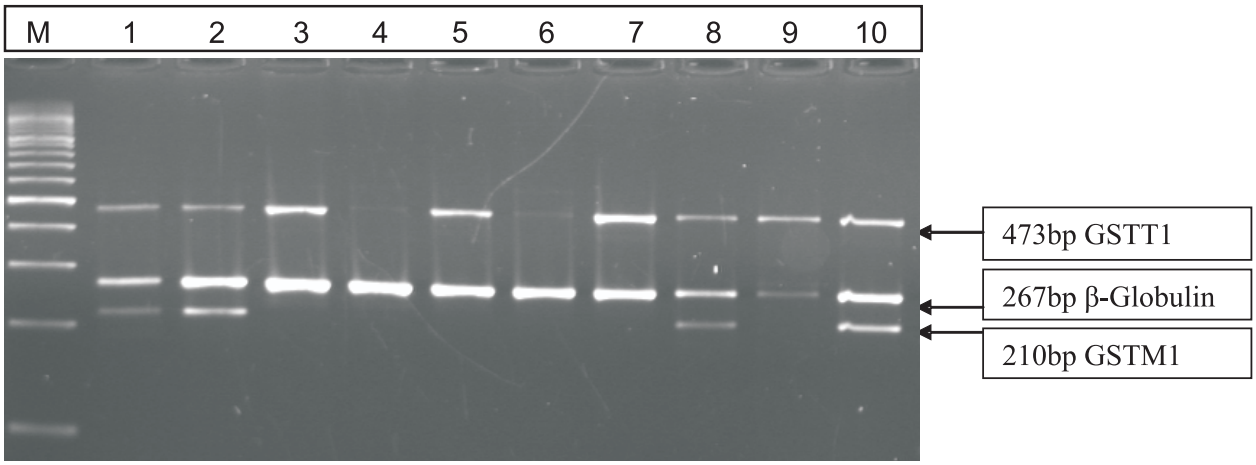


Fig. 4: Multiplex PCR (M: 100 bp marker): Lane 1,2-10 showing presence of both GSTT1 and GSTM 1: Lane3 : GST M1 Null

54 cases and 64 control have been studied for GST polymorphism. Of 54 cases, 13 showed null polymorphism for GSTT, 29 showed null polymorphism for GSTM1 and 6 showed both GSTT1/GSTM1 null polymorphism. Of 64 control samples, 27 showed null polymorphism for GSTT1, 10 showed null polymorphism for GSTM1 and 3 for both GSTT1/GSTM1 null polymorphism. Initial result show frequency of GST null polymorphism is more in cases than controls.

D. HEMATOPOIETIC- LYMPHOID MALIGNANCIES

1. EXPRESSION OF FUSION ONCOPROTEIN AND GENE EXPRESSION PROFILING IN ACUTE AND CHRONIC LEUKEMIA

Scientific Staff	:	Pradeep Singh Chauhan, Dr. Sujala Kapur
Collaborators	:	Dr. Sumita Saluja, Safdarjung hospital
Duration	:	2007 – 2010

Aims, Objective and Background

Diagnosis of chronic myeloid leukemia (CML) is based on the detection of the classic cytogenetic abnormality, BCR-ABL gene or Philadelphia chromosome (Ph chromosome). Fusion proteins with different sizes are encoded depending on the breakpoint in the BCR gene. This translocation t(9;22) is observed in 95% of CML patients, in 2-10% of pediatric AML (acute myeloid leukemia) cases, and in 20-50% of adult ALL (acute lymphoblastic leukemia) cases (Table I). In addition, this translocation may be seen in less than 2% of AML cases and rarely seen in lymphoma and myeloma cases. In general, 3 breakpoint cluster regions in the BCR gene have been described: major (M-bcr), minor (m-bcr), and micro (μ-bcr). BCR/ABL protein varies from 190 KDa to 210 KDa depending upon the breakpoints. Fusion Oncoprotein (Bcr-Abl) is important in signal transduction and cell cycle pathways and is associated with poorer prognosis in ALL which may contribute to chemo-incurability. The current study is designed to understand the significance of presence of fusion oncoproteins in the leukemic cells of patients with acute and chronic leukemias one-step multiplex reverse transcription polymerase chain reaction (RT-PCR**).**

Work done during the period

Flow cytometric Immunophenotyping for the confirmation of diagnosis

FAB diagnosed samples were confirmed by flow cytometric immunophenotyping using a panel of fluorochrome conjugated antibodies [Fluorescein isothiocyanate (FITC) & Phycoerythrin (PE)]. Panel of antibodies included: FITC labeled CD3, CD5, CD7 for T cells; FITC labeled

CD19, PE labeled CD10 for B cells, FITC labeled CD14, CD33 for myeloid cells and CD 34 for immature cells.

RT-PCR Analysis for Bcr-Abl Translocation (p210, p230 and p190)

Isolation of RNA

Leukemic cells were lysed with TRIZOL (Invitrogen) and RNA was recovered according to manufacturer's instructions before resuspension in 10-20 µl of DEPC water. RNA integrity was checked on a 1.2% formaldehyde-agarose gel. cDNA was generated by reverse transcription of total RNA using high capacity cDNA archive kit (Applied Biosystems).

Multiplex RT-PCR for the detection of BCR-ABL Translocation

The BCR/ABL rearrangement was amplified using multiplex RT-PCR using primers for p190, p210 and p230 for the detection of junctions e1a2, b2a2, b3a2. The PCR products were visualized using agarose gel electrophoresis with ethidium bromide staining for 808 bp (normal BCR); 481 bp (e1a2); 385 bp and/or 310 bp (b3a2 and b2a2, respectively). An amplified product from the BCR gene is the only band detected in BCR/ABL negative patients. The presence of this band indicates that the quality of the RNA and the efficiency of cDNA synthesis were good; absence of this band indicates procedural failure (Fig. 1).

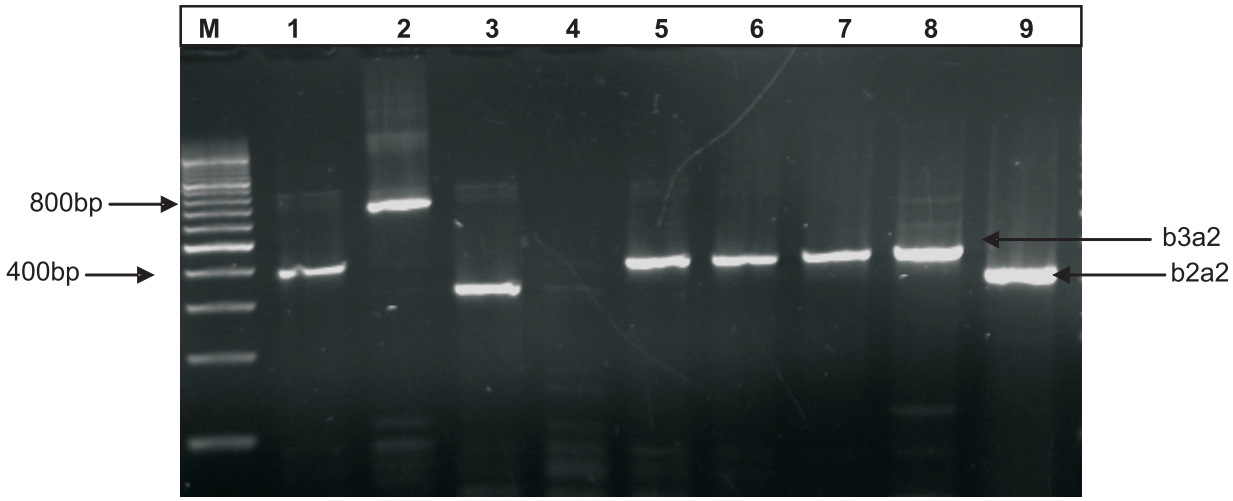


Fig. 1: EtBr stained agarose gel showing the presence of BCR/ABL fusion gene

Flow Cytometric Analysis

Flowcytometric immunophenotyping has been done in 122 samples out of total 128 samples collected to diagnose acute leukemia (AL) as Acute Lymphocytic Leukemia (ALL) and Acute Myeloid Leukemia (AML). Of these, 45 samples were diagnosed as B-ALL, 11 as T-ALL and 61 as of AML and 5 as Biphenotypic Leukemia(BAL) (Table-I).

Table 1: Distribution of acute leukemia cases

Types of Acute Leukemia	No. of Samples
B-ALL	45
T-ALL	11
AML	61
BAL	5

Immunophenotpic analysis of aberrant phenotype in ALL and AML cases

Aberrant expression of myeloid lineage associated antigen (My+ ALL) was seen in 14/56 (25%) cases of ALL while aberrant expression of lymphoid lineage associated antigen (Ly+ AML) was seen in 24/61 (39%) cases of AML. Of 14 samples of My+ ALL, 11/45 (24%) were of My+ B-ALL while 3/11 (27%) were of My+ TALL. Among the Ly+AML cases, CD19 was found in 23% of cases while CD7 was found to be positive in 16% cases (Table 2).

Table 2: Aberrant expression of myeloid markers in ALL patients

CD Markers	Number of Patients (N=61) (%)
CD3	-
CD5	-
CD7	10 (16)
CD10	-
CD19	14 (23)

BCR-ABL in Acute Lymphoblastic Leukemia patient

Forty-four ALL patients (31 male, 13 female) were investigated at presentation. Median age of the patients was 13 years (range 1–57 years). There were 28 children and 16 adults. Thirty-five patients were common ALL (CD10+ve) and 11 were Pre-B-ALL (CD10-ve). All 44 patients were screened for the presence of the *bcr-abl* fusion gene. 13 (29.5%) were found to be positive: Eight were positive for P210 (b3a2/b2a2) and five were positive for P190 (e1a2).

BCR-ABL in Acute Myeloid Leukemia patient

Sixty-Five AML patients (44 male, 20 female) were screened for the presence of the *bcr-abl* fusion gene at presentation. Median age of the patients was 22 years (range 3-85 years). There were 47 adults and 17 children. 8 (12%) were found to be positive: seven were positive for P210 (b3a2/b2a2) and 1 was positive for P190 (e1a2) as shown in Table 3.

Table 3: Distribution of different BCR-ABL fusion transcript in acute leukemia patients

Types of Hybrid transcript	Acute Lymphoblastic leukemia	Acute Myelogenous leukemia
b3a2	1	1
b2a2	7	6
b3a2 + b2a2	-	-
e1a2	5	1

Gene Expression

Profiling RNA was isolated from blood samples (Qiagen RNeasy kit). Quantity was estimated by nanodrop and quality analyzed by agarose gel or by Agilent Bioanalyser. Following labeling, Cy3-labelled cRNA was fragmented and hybridized to the human cancer array chip supplied by M/s Ocimum Biosolutions.

Preliminary results showed up-regulation of MAPKKK cascade, G protein coupled receptor internalization, interleukin-1 beta secretion, regulation of T-cell differentiation, signal transduction by p53 class mediator resulting in induction of apoptosis, regulation of GTPase activity, cell

proliferation and of I-kappaB kinase/NF-kappaB cascade. Down-regulated genes included regulation of caspase activity, oxygen transport, B-cell apoptosis, sodium ion transport and regulation of progression through cell cycle.

2. PREVALENCE AND PROGNOSTIC VALUE OF FLT3 INTERNAL TANDEM DUPLICATION AND D835 MUTATIONS IN ACUTE MYELOID LEUKEMIA

Scientific Staff	:	Pradeep Singh Chauhan, Dr. Sujala Kapur
Collaborators	:	Dr. Sumita Saluja, Safdarjung hospital
Duration	:	2008 – 2011

Aims, Objectives and Background

The wild-type (WT) FLT3 receptor is expressed on hematopoietic progenitor cells and on most in some cases of leukemia, such as AML, chronic myeloid leukemia (CML) in blast crisis, and acute lymphoblast leukemia (ALL). The fms-like tyrosine kinase 3 (FLT3) gene encodes a class III receptor tyrosine kinase and that plays an important role in hematopoiesis. FLT3/ITD and FLT3/D835 are two distinct types of FLT3-activating mutations that have been described in 30% of patients with AML. The majority of FLT3 mutations (20–25%) involved an internal tandem duplication (ITD) in the juxtamembrane domain of the FLT3 (FLT3/ITD) and an additional 5–10% of patients carried a point mutation of Asp835 at exon 20 within the activation loop of the tyrosine kinase domain of FLT3 gene. FLT3/ITD is the most common genetic lesion found in AML patients with normal karyotype and associated with a poor prognosis. Identification of an FLT3 mutation in AML may indicate a need to reassess and modify standard treatment options. In most, but not all studies it is associated with poor clinical outcome. In AML in children, the incidence of FLT3/ITD was reported to be lower than in adults, and it was also associated with poor prognosis. However, there is very little information on its prevalence in India.

Work done during the period

DNA was extracted from bone marrow/peripheral blood samples using a HiPurATM Himedia Blood Genomic Kit. For FLT3-ITD mutation, PCR based amplification of genomic DNA was carried out for exon14 (formerly exon 11). Ten μl of each PCR product was run on 2% agarose gel and visualized under UV light after EtBr staining. The FLT3 wild-type produced a band at 133 bp. Any patient with an additional higher molecular weight band was considered to be positive for an ITD (Fig. 1). Samples showing additional bands, indicative of an ITD, were subjected to sequencing.

For FLT3/D835 mutation, exon 20 of the FLT3 gene where D835 mutation is located, was amplified by PCR. The amplified product was then subjected to digestion with EcoRV restriction endonuclease. In the presence of a wild-type exon (17/20), the amplified fragment was digested into two fragments of 68 and 46 bp. Mutations affecting either D835 or I836 amino acids led to the detection of the undigested product of 114 bp, in addition to the two 68 and 46 bp fragments corresponding to the digestion of the wild-type allele (Fig. 2).

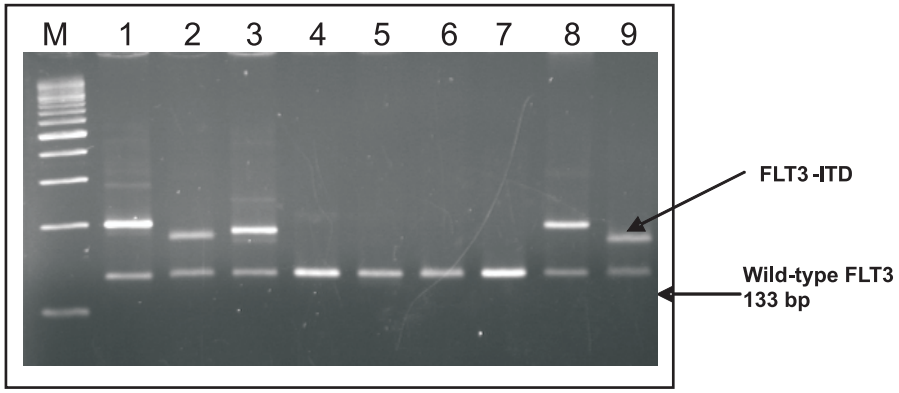


Fig. 1: PCR amplification of genomic DNA for FLT3/ ITD Lane M: 100 bp Ladder Marker. Lane 1, 2,3,8,9, shows the longer product FLT3/ITD in addition to 133 bp wild-type FLT3. The size of the longer product is up to 200 bp. Lane 4 to 7 shows wild type FLT3.

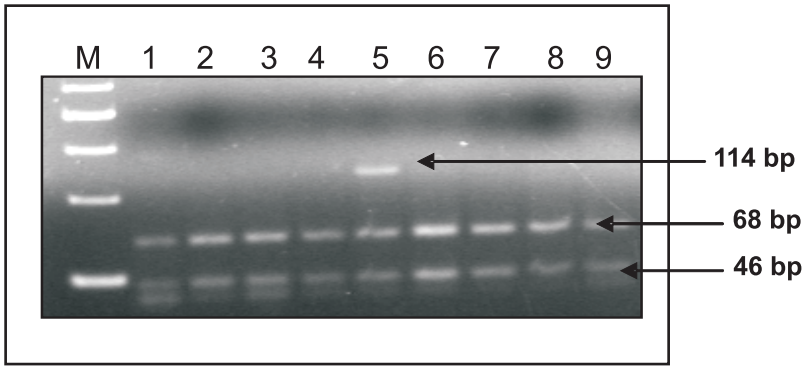


Fig. 2: Detection of D835 mutations in the FLT3 gene. Samples in lane 1-9 (except lane 5) show wild type FLT3. The digestion of amplified products of wild type FLT3 with EcoRV result in appearance of two bands i.e. 68 bp and 46 bp size. Lane 5 shows the undigested bands (114 bp) indicating mutation in 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 31 (23%) patients. These aberrations includes FLT3/ITD in 27 (20%) cases and D835 mutation in 4 cases (3%) which was significantly lower than that of ITD ($P<.001$). In 5 (19%) of the 27 cases with ITD mutation, more than one duplication was detected. 2 cases out of 27 have only one mutant band indicating either homozygous mutation of the allele or the loss of wild type allele for the FLT3/ITD. None of the patients had a combination of FLT3/ITD and D835 mutation in the FLT3 gene

Nucleotide sequencing

Sequencing analysis was carried out in 12 randomly selected 29 samples with FLT3/ITD mutation. All the samples sequenced showed different position of duplications. Sequence analysis revealed all the duplication was in-frame and their length varied from 13 to 59 bp in the juxtamembrane domain of the FLT3 gene. Four of FLT3/ITD cases showed insertion of 5-15 base pairs of nucleotides in addition to duplication while 8 cases showed simple duplication without any insertion or deletion.

Association of FLT3 mutation with clinical features

The clinical and hematologic features of patients with and without FLT3/ITD mutations were compared (Table 1). White blood cell count (WBC) was significantly higher in FLT3/ITD mutated group than without FLT/ITD group ($p<.004$). However, there was no significant difference in the median WBC count, Hb level or platelet count between patients with or without FLT3/D835 mutation. The presence of hepatosplenomegaly or lymphnode involvement was not significantly affected by the FLT3/ITD mutation in the patients. FLT3/ITDs were found more frequently in M5 (23%) followed by M1/M0 (21%) and M2 (20%) FAB subtypes of AML. Significantly higher WBC count were found in FAB M2 ($P<.022$). The frequencies of FLT/ITD mutation did not differ significantly between Auer rod-positive and Auer rod-negative AML cases. FLT3/D835 mutations were found in 4 samples, one each in M1, M2, M4, M5 and AML.

There was no significant difference in response to induction therapy between patients with FLT3/ITD mutation and patients without FLT3/ITD mutation: 67% of the patients with FLT3/ITD and 64% of patients without FLT3/ITD mutation achieved complete response.

3. EXPRESSION OF ACTIVATOR AND TARGET GENES OF NUCLEAR FACTOR-KAPPA B (NF-KB) TRANSCRIPTION FACTOR IN ACUTE LEUKEMIA

Scientific Staff	:	Bharat Bhushan, Dr. Sujala Kapur
Collaborators	:	Dr. Sumita Saluja, Safdarjung hospital
Duration	:	2006– 2009

Aims, Objectives and Background

Abnormalities in the regulation of the nuclear factor-kappa B (NF-kB) pathway are frequently seen in a variety of human malignancies including leukemias, lymphomas, and solid tumors. The majority of these changes are due to alterations in regulatory proteins that activate signaling pathways leading to activation of the NF-kB pathway. Recently, Griessinger E et al (2007) have used a molecule, i.e. AS602868 to inhibit the NF-kB pathway in myeloid leukemic cells. This molecule targets the IKK-α protein and inhibits NF-kB activation thus making it particularly attractive as a new therapeutic approach for AML. The search for similar

molecules that can specifically block the activation of NF-kB pathway and NF-kB-regulated gene expression in other leukemias is continuing.

Work done during the period

NF-kB activation is not uniform among AL patients. In the current study, genes that may be potential targets for inhibiting the NF-kB pathway such as *IkB-α, IKK-α, IKK-β, IKK-γ, P53, Cyclin D1, Bcl-2, cIAP-2, Survivin and Traf-2* are being analyzed. Real-time quantitative PCR was carried out in ABI 7000 SDS with the c-DNA as template using TaqMan probe assay. Delta delta C_T ($\Delta\Delta$ C_T) Method was used to analyze the Real Time RT-PCR data. Using the $2^{-\Delta\Delta C_T}$ method, the data was presented as the fold change in the target gene expression in leukemia normalized to an internal control gene and relative to the calibrator (normal bone marrow).

Of 58 samples, 17 samples were diagnosed as B-ALL, 3 samples as T-ALL and 38 samples as AML. So far, 35 samples have been analyzed (20 AML & 15 ALL) for Real Time PCR. Activation of NF-kB pathway was determined by the expression level of Ik-B gene. 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.

NF-kB pathway was found to be activated more often in AML as compared to ALL (84.2% and 40% respectively). In AML, frequency of NF-kB activation was similar between children and adults (85.7 and 83.3% respectively). However, in ALL, activation of NF-kB pathway was found to be significantly higher in children compared to adults (62.5% and 16.6% respectively) (Fig. 1).

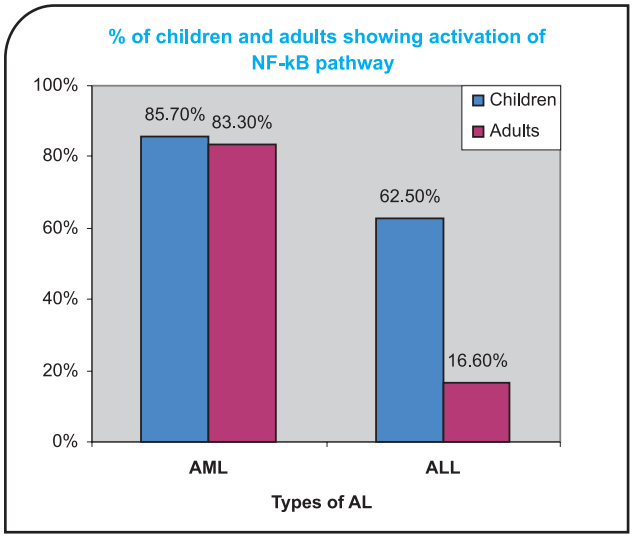


Fig. 1: NF-kB Activation in childhood and Adult AL.

Gene expression in acute leukemia

IkB-α was significantly higher in AML compared to ALL samples (7.8 ± 8.6 and 2.2±0.4 fold respectively, p=0.02). In addition, significant differences in expression level of IKK-B, P53, cIAP-2 and SURVIVIN were found in samples of AML and ALL (Table 1).

Table 1: Gene Expression in AML vs ALL

Genes	Gene Expression Level (Mean ± SD)		p-value
	AML	ALL	
IkB-α	7.8 ± 8.6	2.2 ± 0.4	0.02*
IKK-A	1.1 ± 0.5	1.4 ± 0.3	0.1
IKK-B	0.9 ± 0.3	1.3 ± 0.5	0.04*
IKK-G	0.6 ± 0.3	0.9 ± 0.4	0.1
P53	0.8 ± 0.3	1.2 ± 0.2	0.01*
cIAP-2	4.6 ± 3.4	2.6 ± 1.3	0.05*
BCL-2	2.0 ± 2.0	2.1 ± 1.1	0.9
SURVIVIN	0.1 ± 0.08	0.18 ± 0.06	0.06*
CYCLIN D1	0.5 ± 1.1	0.47 ± 0.56	0.8
TRAF-2	0.6 ± 0.2	0.8 ± 0.2	0.4

Gene expression in responders and non-responders of AML

Ik-B expression was found to be higher in responders (6.3 ± 6.3) compared to non responders (3.1 ± 2.4) (p=0.4), however, the difference was statistically not significant. In responders, adults had lower expression of IkB (2.7±1.0 fold) compared to children (9.1±7.6 fold). No significant difference was seen in the expression level of IKK-A, IKK-B and IKK-G between responder and non-responder of AML patients. Expression level of P53 was significantly lower in non-responder group of AML patients while the expression level of the cyclin D1 was found to be higher in non-responder (2.2 ± 1.5) compared to responders of AML (0.07 ± 0.09), however, no statistical significant difference was seen between the groups (p=0.2).

Gene expression in responders and non-responders of ALL

In contrast to AML, no significant difference was found in the expression level of IkB-α in responder (2.0 ± 0.1) and non-responder group of ALL (2.2 ± 0.6) (p=0.5). In addition, there was no significant difference in the expression level of IKK-A, IKK-B and IKK-G between responders and non-responders of ALL patients. Expression level of Bcl-2 was found to be higher in non-responder group versus responder group (3.1±0.9 vs. 1.9±0.6, p=0.1), although

the difference was not significant. However, expression level of cIAP-2 was significantly lower in non-responder group of ALL patients compared to responders (1.1± 0.07 vs. 2.9 ± 0.5, p=0.02).

Role of CD34 marker in NF-kB activated samples

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. No significant difference seen in the expression level of other studied genes in AML and ALL.

Correlation of P53 and IKK-alpha gene expression in non-responders of AML

Loss of p53 function leads to transcriptional induction of IKK-α which results in NF-kB mediated gene expression. In this study, expression level of P53 was found to be significantly lower in non-responders compared to responders of AML (0.6 ± 0.3 and 1.0 ± 0.1 respectively, p=0.02) (Table-III). The expression of IKK-α gene was inversely correlated with the expression of P53 (correlation coefficient (r) = -0.9, (Fig.3).

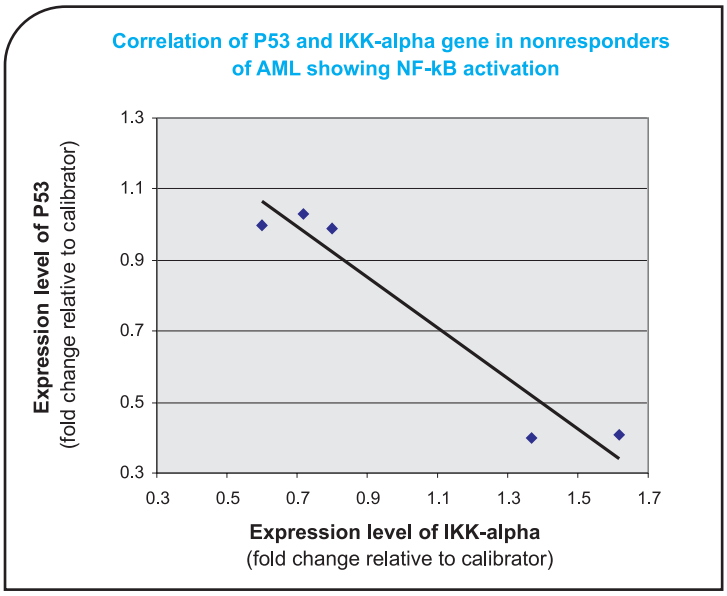


Fig. 3 : Correlation of P53 and IKK-α gene in non-responders of AML

Preliminary results indicate that the dysregulation of NF-kB signaling was associated with upregulation of cell survival genes (cIAP-2 and Bcl-2) rather than cell proliferation gene (CyclinD1) in both ALL and AML samples indicating that targeted therapy to downregulate cell survival genes may be beneficial in these cases.To get clearer picture and understand the role of this pathway in the pathogenesis of AL, adults and children will be analyzed separately as the blasts cells of children and adults have different biology, behaviour and prognosis. More samples (15 AML, 19 ALL) will be collected for statistical analysis.

4. IDENTIFICATION OF DIAGNOSTIC AND PROGNOSTIC BIOMARKERS IN BRAIN TUMORS: A TISSUE MICROARRAY BASED APPROACH

Scientific Staff	:	Dr. Avninder Singh, Dr. Sunita Saxena
Collaborators	:	Dr. Karam Chand Sharma, Safdarjung hospital Dr. Stephen Hewitt, TARP laboratory, National Cancer Institute, NIH, Bethesda, USA
Duration	:	2008 – 2009

Aims, Objectives and Background

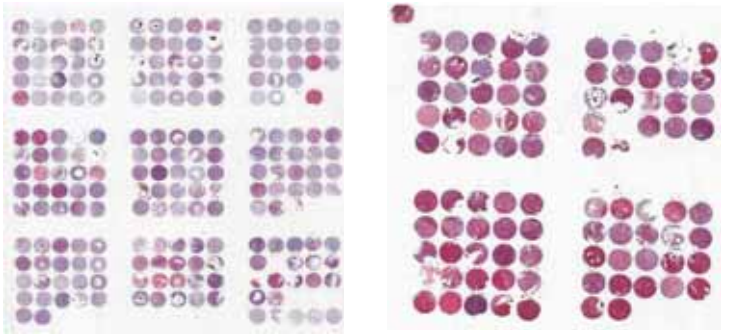
Tumors of the central nervous system are one of the leading causes of cancer morbidity and mortality and remain difficult to cure despite advances in surgery and adjuvant therapy. Unfortunately, apart from routine histological classifications and WHO grading system, our ability to effectively stratify 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.

New high throughput technologies like cDNA microarray have greatly facilitated the simultaneous analysis of thousands of genes and have aided in identifying differentially expressed genes. Large-scale gene expression profiling approach will help in identifying novel molecular signatures and pathways driving the various stages of tumor progression. Such molecular profiling may eventually lead to targeted therapeutic approaches that could improve the clinical outcome of identification of these patients. To provide conclusive evidence for the involvement of a molecular alteration, it is necessary to analyze hundreds of tumor samples. 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, analyzes a large number of samples and improves statistical precision in addition to enhanced speed and quality of analysis.

The aims were 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 and to study the protein expression of the differentially expressed genes identified by cDNA microarray at National Cancer Institute using immunohistochemistry TMA-IHC.

Work done during the period

TMA of 300 tumors were constructed into 2 blocks of 1 mm core size and stained for under-mentioned target antigens for analysis. A panel of 24 primary antibodies were selected for studying their immunohistochemical expression. On two separate TMAs i.e; glioma and schowannoma-meningiome, statistical analysis of these marks will be done to identify biomarkers of diagnostic / prognostic significance.



Glioma TMA

Meningioma-Shwannoma-TMA

Fig.: TMA slides- H & E stain

Future plan

To do tissue transblotting from the glioma TMA sections for proteomic studies.

PATHOLOGY
OF
INFECTIOUS DISEASES

CHLAMYDIASIS

1. ROLE OF CHLAMYDIAL HEAT SHOCK PROTEINS IN PATHOGENESIS OF GENITAL TRACT INFECTION IN WOMEN

Scientific Staff	:	Rajneesh Jha, Dr. Aruna Singh
Collaborators	:	Dr. Sudha Salhan, Safdarjung hospital
Technical Staff	:	Mrs. Madhu Badhwar, Mrs. Asha Rani
Duration	:	2006-2009

Aims, Objectives & Background

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 women. Studies on cHSP60 and cHSP10 specific mucosal and peripheral proliferative responses have suggested a probable role of cHSPs in modulation of mucosal immune responses. Overall previous studies suggest that cHSPs specific immune responses play an important role in immunopathogenesis associated with chlamydial infection. However, there is no study available wherein the expression of cHSP60 and cHSP10 at the actual site of infection has been assessed. Therefore the objective of the present study was to examine the possible relationship between cHSP60 and cHSP10 expression and damaging sequelae of *C. trachomatis* infection such as infertility.

Work done during the period

In this study, the cHSP60 and cHSP10 mRNA and protein expression were analyzed in cervical cells in fertile and infertile women that have previously encountered *C. trachomatis*.

Study population

Based on routine laboratory diagnosis (DFA and PCR), the cervical lavage samples from 14 *C. trachomatis* positive female patients were collected and divided into two groups. The fertile group (n=7) consisted of *C. trachomatis* positive women with no infertility problems and without any clinical history of past infection; the infertile group (n=7) comprised of *C. trachomatis* positive women with infertility and who had clinical history of past infection for more than three years. The age range of women in each group were between 23 and 43

years. None of the enrolled patients had bacterial vaginosis or were co-infected with *Candida* sp., *T. vaginalis*, *M. hominis*, *U. urealyticum* or *N. gonorrhoeae* in the cervix. All samples included in the study had viable cells as well as homogenous in nature and counted more than $1x10^5$ epithelial cells/ml.

Relative expression of cHSP60 and cHSP10 genes in *C. trachomatis* infected cervical epithelial cells

To investigate whether mRNA expression of cHSP60 and cHSP10 genes might be different between 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).

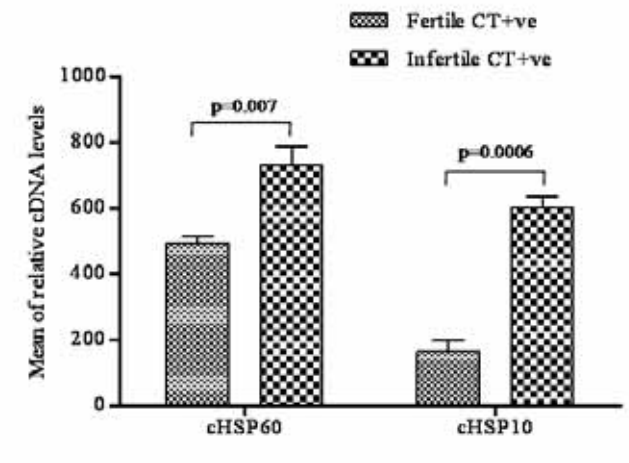


Fig. 1: Real time RT-PCR assay targeting relative transcript levels from the cHSP60 and cHSP10 genes in RNA/cDNA prepared from *C. trachomatis* infected cervical epithelial cells from each of the seven fertile and seven infertile women. Data shown were normalized to the level of *C. trachomatis* 16S 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.

Flow cytometric analysis of intracellular levels of cHSP60 and cHSP10 in *C. trachomatis* infected cervical epithelial cells

To investigate 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 (Fig. 2). 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. 3).

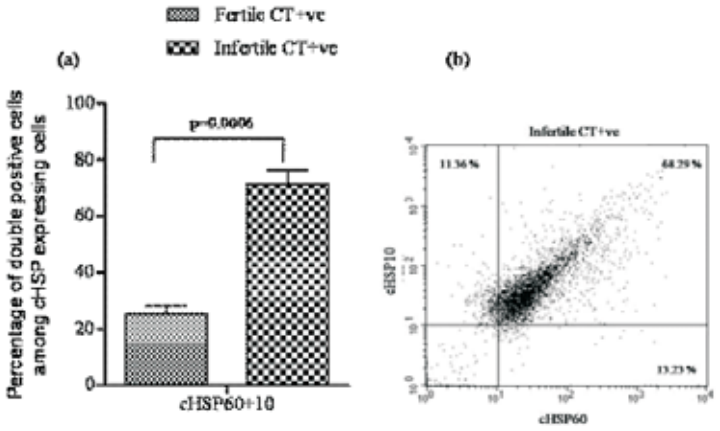


Fig. 2: Flow cytometric assay targeting intracellular levels of cHSP60 and cHSP10 in *C. trachomatis* infected cervical epithelial cells from each of the seven fertile and seven infertile women. Cells were simultaneously stained with monoclonal antibodies of cHSP60 and cHSP10 conjugated with FITC and 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.

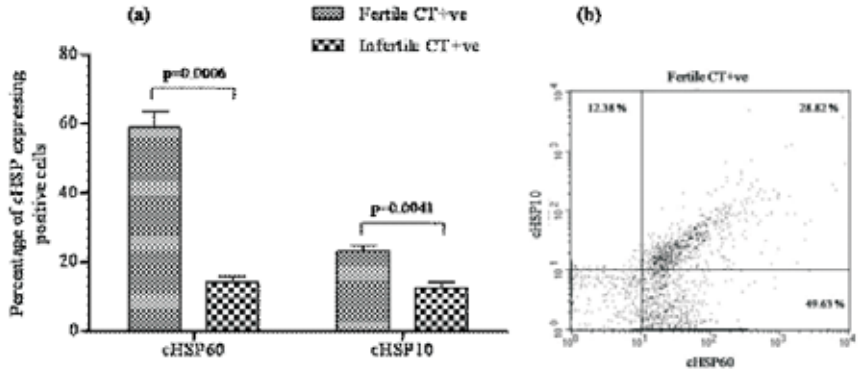


Fig. 3: Flow cytometric assay targeting intracellular levels of cHSP60 and cHSP10 in *C. trachomatis* infected cervical epithelial cells from each of the seven fertile and seven infertile women. Cells were simultaneously stained with monoclonal antibodies of cHSP60 and cHSP10 conjugated with FITC and 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 double positive cells (both cHSP60 and cHSP10) among cHSP expressing cells. Bars represent standard error. (b) Representative figure (dot plot) showing intracellular levels of cHSP60 and cHSP10 in infected cervical epithelial cells of infertile women.

Conclusion: 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. Thus these cHSPs could be possible drug target for disease management and also points out that further research is warranted to more precisely define the potential contribution of these antigens to the immunopathologic process associated with chlamydial infection.

2. EFFECT OF SEX HORMONES ON INDUCTION OF IMMUNITY BY DENDRITIC CELLS IN FEMALE REPRODUCTIVE TRACT DURING CHLAMYDIA TRACHOMATIS INFECTION

Scientific Staff	:	Tanvi Agrawal, Vikas Vats, Dr. Aruna Singh
Collaborators	:	Dr. Sudha Salhan, Safdarjung hospital Dr. Paul Wallace, Roswell Park Cancer Institute, Buffalo, USA
Technical Staff	:	Mrs. Asha Rani, Mrs. Madhu Badhwar
Duration	:	2005- 2010

Aims, Objectives & Background

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*.

Work done during the period

Cervical lavage samples were collected from symptomatic female patients (age range: 20-40 years) attending the Gynecology Outpatient Department of Safdarjung hospital, New Delhi, India. For studying the role of dendritic cell subsets in modulating immune response to chlamydial infection, women were characterized into four groups on the basis of their clinical history: Group I (n = 28) comprised of 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 of *Chlamydia*-positive women with MPC (number of PMNLs >30) and *Chlamydia* positive women with fertility related disorders (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 women with MPC and FD had pDC in their cervical samples. The median and range of absolute numbers of pDCs/cervical sample are given in Table 1. 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: Absolute median number of myeloid and plasmacytoid dendritic cells in cervical lavage samples

MYELOID DENDRITIC CELLS			
Control	**CT positive asymptomatic**	**CT positive with MPC**	**CT positive with FD**
36 (0-1256)	**1788 (0-40170)[a]**	**720 (0-10444)[a,b]**	**1856 (21-8473)**
PLASMACYTOID DENDRITIC CELLS			
Control	**CT positive asymptomatic**	**CT positive with MPC**	**CT positive with FD**
13 (0-1200)	**1750 (0-33840)[a]**	**3890 (1162-80752)[a]**	**3783 (54-10845)**

Note: MPC = Mucopurulent Cervicitis, FD = Fertility Disorders

Dendritic cell population after resolution of infection

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 (Fig. 1). The median number of pDCs was lower in both the *Chlamydia*-infected groups after therapy.

Correlation of chlamydial infectious load with immune factors

For studying the correlation of chlamydial infectious load with immune factors as dendritic cells, *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 (Table 2). 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 (Table 3). This data suggests that clinical condition presented is decided by interplay of infectious load and host immune responses.

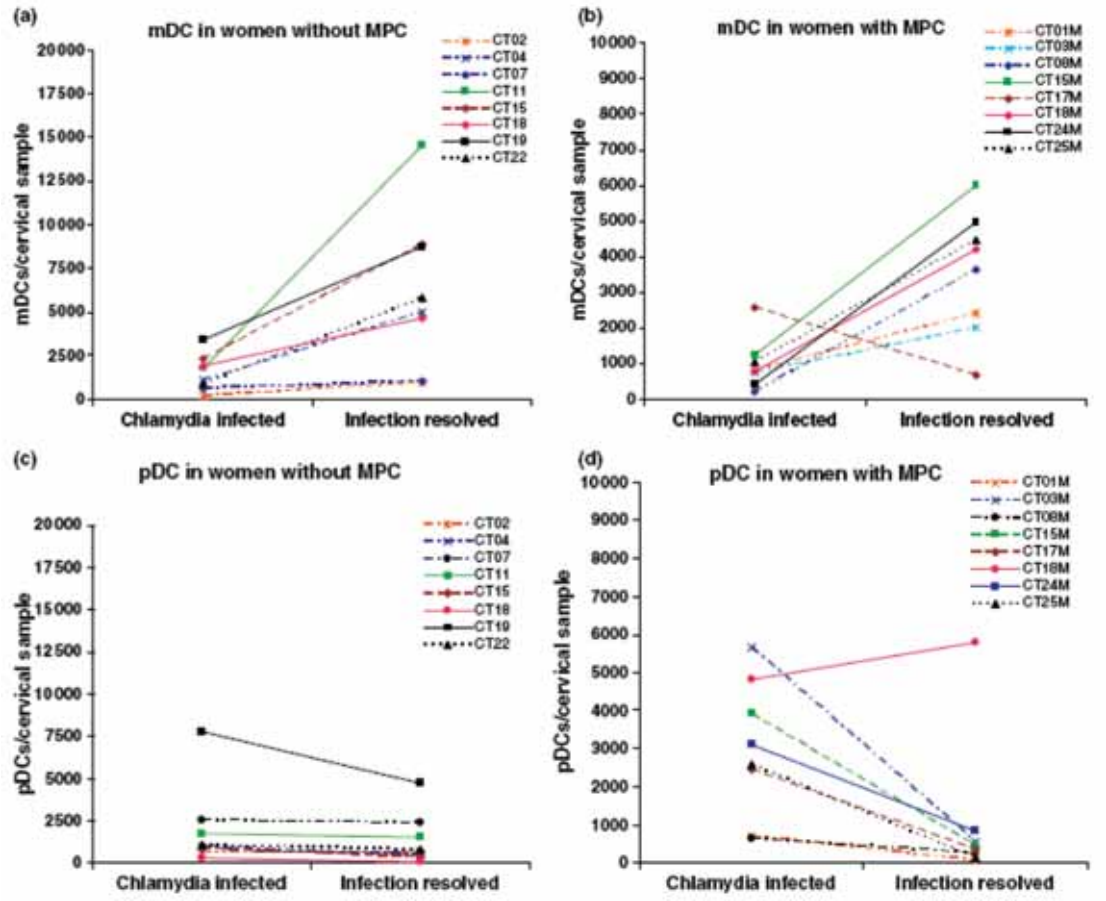


Fig 1: Change in number of dendritic cells (DCs) in cervical samples of *Chlamydia*-positive women without or with mucopurulent cervicitis (MCP) after resolution of chlamydial infection. (a, b) Myeloid DCs (mDCs) in cervical samples of eight women with or without MCP who returned for follow-up after resolution. (c, d) Similar data for plasmacytoid DCs (pDCs) in cervical samples.

Table 2: Distribution of chlamydial inclusion counts in *C. trachomatis* positive women

IFUs	*Chlamydia* positive asymptomatic women (n=127)	*Chlamydia* positive with MPC (n=86)	*Chlamydia* positive with FD (n=108)
$<10^1$	4	1	20
10^2	25	7	49
10^3	30	29	13
10^4	38	47	12
$>10^4$	3	16	6

Note: Data expressed as percentage of women having IFU in particular range, IFUs; Infection forming units mL^{-1} of transport media, MPC; Mucopurulent Cervicitis

Table 3: Correlation of Infection Forming Units (IFUs) with various immune factors

Immune parameter	Asymptomatic *Chlamydia* positive (n=127)	*Chlamydia* positive with MPC (n=86)	*Chlamydia* positive with fertility disorders (n=115)
CD4	0.57#	0.63#	0.70#
CD8	-0.78#	0.72#	-0.80#
CD14	0.49#	0.38	0.32
mDC/cervical sample	0.72#	0.31	0.28
pDC/cervical sample	0.16	0.85#	0.61#
IL-1β	0.11	0.77#	0.10
IL-2	0.75#	0.02	0.29
IL-6	0.27	-0.01	0.42
IL-8	-0.84#	0.79#	-0.57#
IL-10	0.42	0.74#	0.83#
IL-12	0.62#	0.23	-0.16
IFN-γ	0.58#	0.82#	-0.48#
β-estradiol	0.37	0.57#	0.65#
CRP	0.15	0.59#	-0.71#
CTR MOMP IgA	0.66#	0.49#	0.62#
cHSP60 IgA	0.04	0.18	- 0.79#
cHSP10 IgA	0.12	0.41	- 0.81#

Note: Data are spearman's correlation coefficients obtained between various immune parameters and chlamydial IFUs. #P<0.05 by spearman correlation coefficient. IL; interleukin, IFN; interferon, CTR; *Chlamydia trachomatis*, cHSP; chlamydial heat shock protein

3. MODULATORY ROLE OF ANTICHLAMYDIAL AGENTS IN CHLAMYDIA TRACHOMATIS INFECTION AND THEIR THERAPEUTIC POTENTIAL

Scientific Staff	:	Pragya Srivastava, Apurb Rrashmi Bhengraj, Dr. Aruna Singh
Collaborators	:	Dr. Sudha Salhan, Safdarjung hospital
Technical Staff	:	Mrs. Madhu Badhwar, Mrs. Asha Rani
Duration	:	2007 -2010

Aims, Objectives & Background

During chlamydial infection in animals and humans as well as in cell culture systems, a wide array of inflammatory cytokines have been implicated to contribute towards *Chlamydia*-induced pathologies. Hence apart from antibacterial activity, its treatment may require modulatory effect on cytokines to clear the pathology associated with *C. trachomatis*. Therefore in the present study, our aim was to elucidate if azithromycin also modulates the production of cytokines in the process of eradication of infection.

Work done during the period

We investigated the levels of interleukin (IL)-1beta (1β), IL-2, IL-4, IL-6, IL-8, IL-10, IL-13, interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α) by ELISA in women representing different clinical conditions, i.e. *Chlamydia*-positive fertile and infertile women with genital tract infection of *C. trachomatis* before and after treatment with azithromycin. 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). In serum, decrease in levels of IL-8 (P =0.04) was observed in Group III after therapy (Table 2). Overall, our data suggests that azithromycin may inhibit the secretion of cytokines and hence play an anti-inflammatory role which could contribute to its clinical efficacy suited for the treatment of chlamydial infection in immunopathological conditions like infertility.

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

	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*

* 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

Table 2: Cytokine concentration in serum before and after treatment

	Group II (n=20)			Group III (n=18)		
	Before treatment	After treatment	P value	Before treatment	After treatment	P value
IL-1β (pg/ml)	4.5 (UDL-14)	5.8 (UDL-20)	0.3	4.5 (UDL-18)	5 (UDL-15)	0.2
IL-2 (pg/ml)	4.4 (UDL-8)	5 (UDL-10)	0.5	4 (UDL-8)	4.6 (UDL-8)	0.3
IL-6 (pg/ml)	3.7 (UDL-12)	3 (UDL-14)	0.8	4 (UDL-10)	4.5 (UDL-12)	0.6
IL-8 (pg/ml)	12.5 (UDL-94)	8.5 (UDL-66)	0.1	23 (3-142)	17 (7-96)	0.04*
IL-10 (pg/ml)	4.4 (UDL-8)	3.5 (UDL-8)	0.5	3.6 (UDL-9)	4 (UDL-12)	0.6
IL-13 (pg/ml)	4.5 (UDL-11)	4.5 (UDL-12)	0.9	5 (UDL-8)	4.8 (UDL-10)	0.5
IFN-γ (pg/ml)	10 (UDL-28)	12 (UDL-30)	0.3	8 (UDL-18)	9 (UDL-34)	0.3
TNF-α (pg/ml)	8.5 (UDL-39)	6.5 (UDL-28)	0.5	6.8 (UDL-26)	8 (UDL-36)	0.4

* 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

Scientific Staff	:	Apurb Rashmi Bhengraj, Pragya Srivastava, Dr. Aruna Singh
Collaborators	:	Dr. Sudha Salhan, Safdarjung hospital
Technical Staff	:	Mrs. Madhu Badhwar, Mrs. Asha Rani
Duration	:	2007-2010

Aims, Objectives & Background

C.trachomatis has been historically sensitive to the tetracyclines, macrolides, and fluoroquinolones. Recent reports have noted increasing *in vitro* resistance. The clinical significance of these findings is unknown. Hence, it would be important 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 other options such as topical microbicide would be useful to control chlamydial infections. Talwar et al have recently developed a polyherbal cream, BASANT, for topical use which has been formulated employing a combination of active compounds such as, Aloe vera (2.5% w/v), Amla (2.5% w/v), Curcumin (0.36% w/v) and Reetha saponins (1% w/v). So, in this study our objective was to determine the drug sensitivity profile of *C.trachomatis* isolates from patients with treatment failure and recurrent infection with the first line drugs as well as with the new polyherbal cream.

Work done during the period

During the period under report, a total of 21 isolates have been successfully isolated from the patients. These *C. trachomatis* isolates were passaged several times for increasing the inclusion forming units and preserved at -80°C.

In vitro susceptibility assay

The doxycycline drug sensitivity assay was performed on the five isolates using cell culture method for Minimum Inhibitory Concentration (MIC) determination.

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	-	-

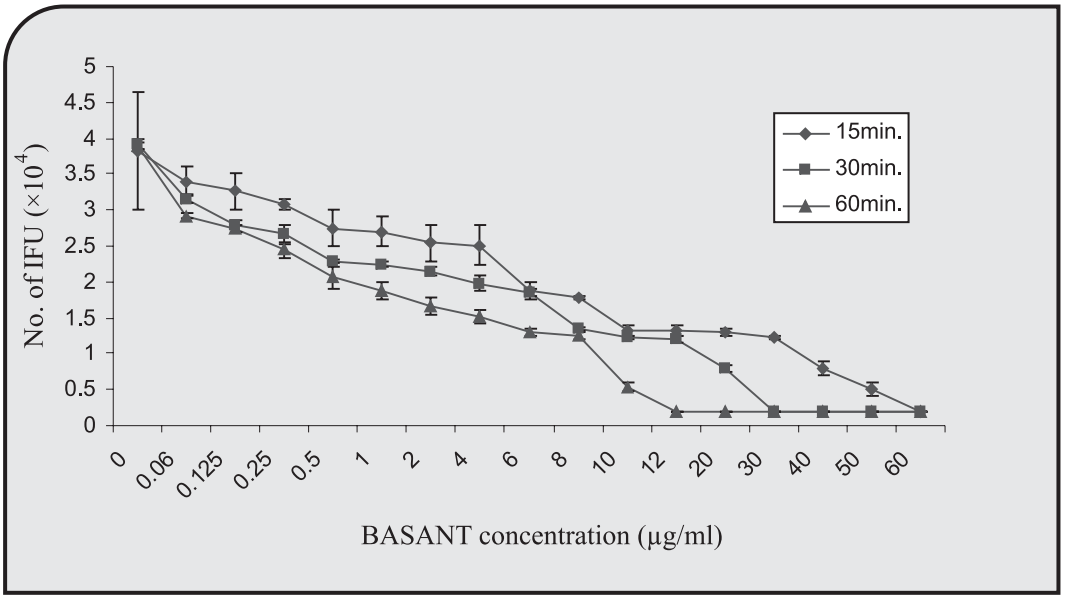
The isolates of *C. trachomatis* had shown different susceptibility profile. One of the isolates could not reach the MIC 90, hence, it could be suspected for a heterotypic resistant isolate.

In vitro susceptibility assay for BASANT

Two methods were employed to determine in vitro sensitivity of *C.trachomatis* towards BASANT, viz.: 1) Pre-infection incubation and 2) Post-infection incubation.

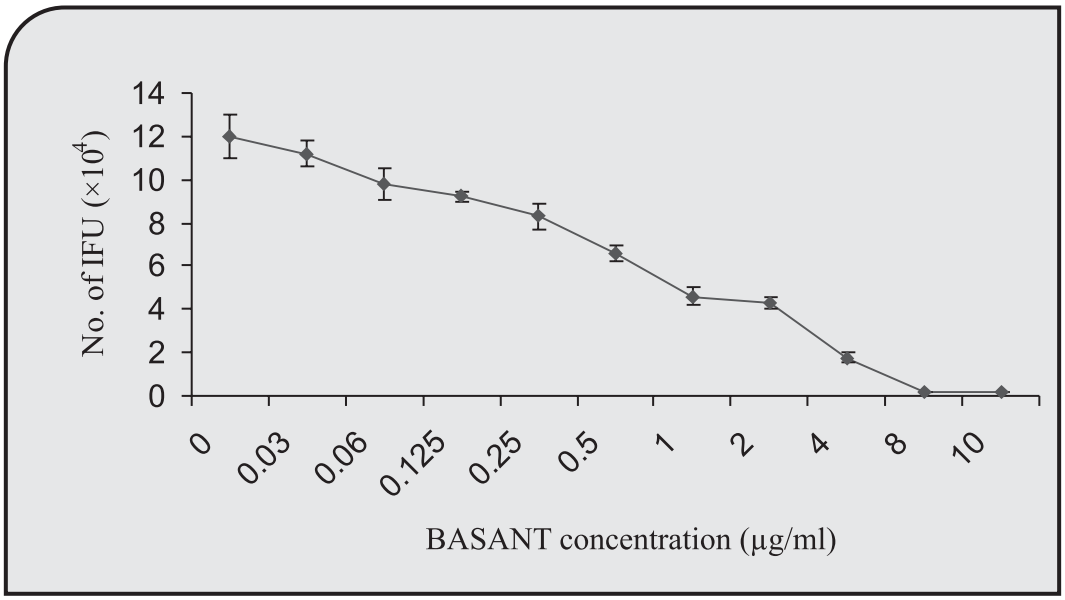
1) Pre-infection incubation with BASANT

Elementary bodies were pre-incubated with BASANT to mimic its topical action. Further, these were infected into host HeLa 229 cells and MIC was determined by direct immunofluorescence assay.



2) Post-infection incubation

Elementary bodies of *C. trachomatis* were infected into host cells and treated with BASANT. After 40 hours of infection MIC was determined by direct Immunofluorescence assay and found that BASANT has its inhibitory activity inside host cells.



Activity of BASANT was also determined for clinical isolates of *C. trachomatis* and it has demonstrated antimicrobial activity against *C.trachomatis* with standard as well as clinical isolates. This implies the potential clinical utility of BASANT for the prevention of *C.trachomatis* infection.

5. ROLE OF *CHLAMYDIA PNEUMONIAE* IN CORONARY ARTERY DISEASE (CAD) PATIENTS

Scientific Staff	:	Hem Chandra Jha, Dr. Aruna Singh
Collaborators	:	Dr. Jagdish Prasad, Safdarjung hospital
Duration	:	2006-2010

Aims, Objectives & Background

Atherosclerosis is an inflammatory disease which may be the outcome of responses to microbial antigens. Several conserved components of the bacterial cell wall have been shown to bind to receptors on the cell surface of monocyte and macrophages which may induce production of pro-inflammatory cytokines. 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. Many pathogens have been implicated in the development of CAD including *C. pneumoniae*, *Helicobacter pylori (H. pylori*) and Cytomegalovirus (CMV). In recent years, attention has been broadened to include the burden of infection hypothesis, the concept that it is not one specific infection per se but the cumulative burden of infection that increases a person's risk of CAD. It has also been suggested that increasing numbers of infectious pathogens were significantly related to the extent of atherosclerosis and multiple pathogens may augment the risk conveyed by one pathogen.

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, assess the 'p' value and odds of CAD with increasing burden of infection and their association with the level of hs-CRP**.** We also assessed the association between these pathogens and conventional risk factors among CAD patients in India, such as sex, smoking, alcohol intake, genetic predisposition, hypertension, and diabetes mellitus and do they impose an additional independent risk for the presence and severity of CAD. Additionally, CAD-patient first degree relatives (CAD-R) were also analysed for seropositivity to *C. pneumoniae*, *H. pylori*, CMV and hsCRP. We also evaluated these cytokines (IL-6, IL-2 and TNF- α) in CAD patients positive for *C. pneumoniae* IgA with low, medium and high levels as it will help in understanding the chronicity of CAD in India. In addition, taking IL-6 as a marker in CAD patients, baseline and

physiological characteristics were compared with controls in order to elucidate its significance in disease progression. Further to establish IL-6 as an independent atherosclerotic marker, we evaluated correlation between levels of IL-6 and hsCRP, and levels of IL-6 and IL-2 in CAD patients. Moreover, there is no study where levels of these plasma circulatory markers (IL-2, IL-4, IL-8, IL-10, IL-13, TNF-α, IFN-λ, ICAM-1, VCAM-1) in *C. pneumoniae* IgA positive and hsCRP positive groups of CAD patients have been evaluated which is required for understanding pathogenesis of CAD. Hence this study perceives the levels of plasma circulatory inflammatory markers in CAD patients in presence of well established CAD markers, *viz*: *C. pneumoniae* IgA and hsCRP in Indian population.

Work done during the period

For 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 a performed using an ELISA kit as per manufacturer's instructions. Level of the hsCRP (more than 3mg/l) in serum was considered as hs-CRP positive in dichotomized result. Serum was separated within 2 hours of blood collection and kept at -80°C until used for detection of hsCRP, IL-2, IL-4, IL-6, IL-8, IL-10, IL-13, TNF-α, IFN-λ, ICAM-1 and VCAM-1.

Seropositivity in patients and healthy controls

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 patients 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 was also significantly high in CAD patients as compared to controls (32.3% v/s 19.8 %; P = 0.010). Presence of hs- CRP was also significantly high in CAD patients as compared to controls and % positivity was (59.4 v/s 31.3) (P <0.001) (Table 1).

Seropositivity in combined infection group in CAD patients

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) (Table2). 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 (Fig1). However, none of the groups showing significant differences with other groups.

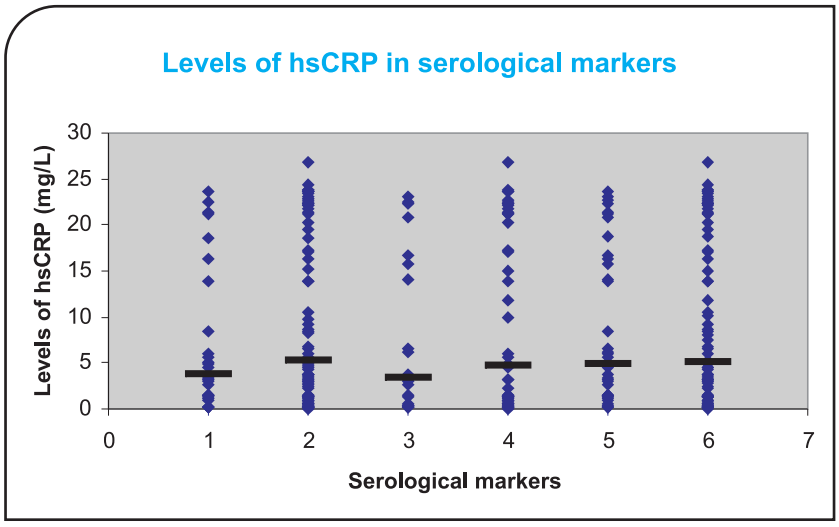


Fig. 1: Levels of high sensitive C - reactive protein according to serologic markers in CAD patients
On Y-axis: Levels of hs- CRP in serological groups.
On X-axis: 1=C. pneumoniae IgG CAD patients group, 2= C. pneumoniae IgA CAD patients group, 3=H. pylori IgG CAD patients group, 4=H. pylori IgA CAD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

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

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*

*Statistically significant

Table 2: *Helicobacter pylori* and *Chlamydia pneumoniae* seropositivity in CAD patients

	C. pneumoniae IgG(+ve)	*C. pneumoniae* IgG(-ve)	*C. pneumoniae* IgA(+ve)	*C. pneumoniae* IgA(-ve)
***H. pylori* IgG(+ve)**	**32(16.7)**	45(23.4)	***71(37)**	06(3.1)
***H. pylori* IgG(-ve)**	39(20.3)	76(39.6)	72(37.5)	43(22.4)
***H. pylori* IgA(+ve)**	**46(24)**	55(28.7)	***92(47.9)**	09(4.7)
***H. pylori* IgA(-ve)**	25(13)	66(34.4)	51(26.6)	40(20.9)

*Significant, % in parenthesis, +ve=positive, -ve=negative.

Conventional risk factors

All these conventional risk factors were evaluated with serological groups in terms of odds ratio (O.R.) (Fig. 2). Males were more often detected seropositive for *C. pneumoniae*-IgA and *H. pylori*-IgA in CAD positive patients (O.R. =1.3 and 1.2) respectively. Heavy smokers among CAD patients were more positive for *C. pneumoniae*-IgG, *C. pneumoniae*-IgA, *C. pneumoniae*-IgM, *H. pylori*-IgM and hs-CRP (O.R. =1.5, 2.4, 2.0, 1.7 and 1.5) respectively. However, occasional smokers were found more positive for *C. pneumoniae*-IgM and H. *pylori*-IgG seropositive group (O.R. =1.8 and 2.3) respectively. In addition, occasional alcoholics were seropositive for *H. pylori*-IgG, *H. pylori*-IgM and CMV-IgG (O.R. =2.3, 1.9 and 1.7) respectively. Similarly, non-alcoholics were found more seropositive for *C. pneumoniae*-IgA, *H. pylori*-IgA and hsCRP group (O.R. =1.4, 1.2 and 1.6) respectively. Patients with family history of CAD, BP and diabetes were more associated with *H. pylori*-IgG, *H. pylori*-IgM, CMV IgG and *C. pneumoniae*-IgA, *C. pneumoniae*-IgM, *H. pylori*-IgG, CMV-IgM and *C. pneumoniae*-IgA, *H. pylori*-IgM, CMV-IgM, respectively.

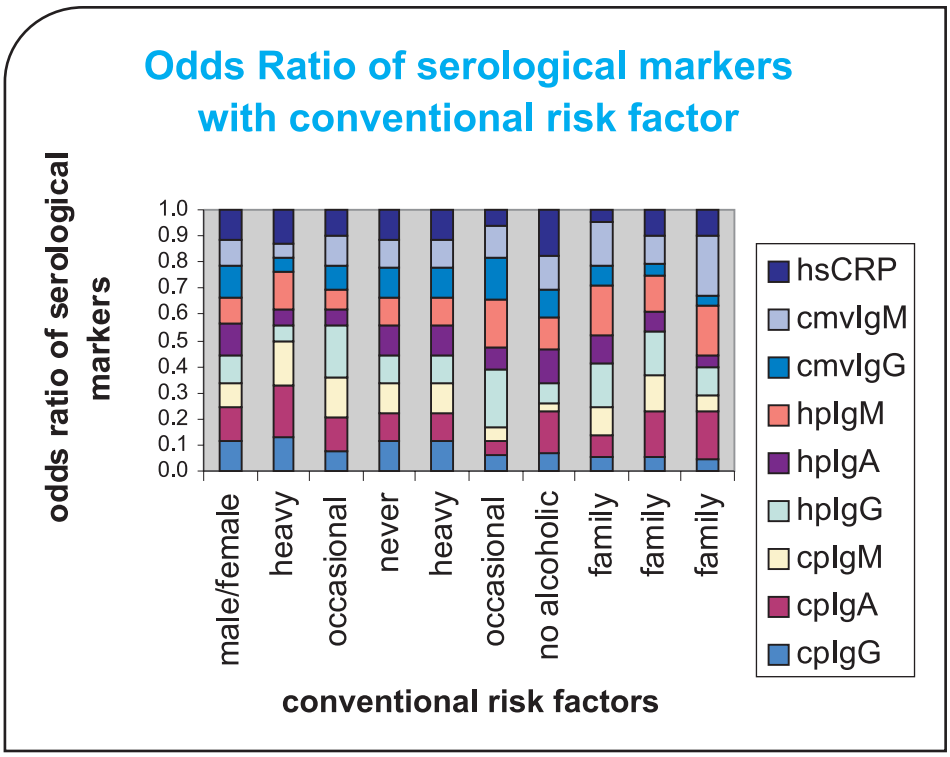


Fig. 2: Odds ratio of serological markers with conventional risk factors.
On Y-axis: odds ratio (odds of CAD patients/odds of control) of serological markers.
On X-axis: conventional risk factors including in this study.
Males CAD patients seropositive for *C. pneumoniae*-IgA and *H. pylori*-IgA (O.R. =1.3 and 1.2) respectively. Heavy smokers CAD patients were more positive for *C. pneumoniae*-IgG, *C. pneumoniae*-IgA, *C. pneumoniae*-IgM, *H. pylori*-IgM and hs-CRP (O.R. =1.5, 2.4, 2.0, 1.7 and 1.5) respectively. However occasional smokers were found more positive for *C. pneumoniae*-IgM and *H. pylori*-IgG seropositive group (O.R. =1.8 and 2.3) respectively. Occasional alcoholics were seropositive for *H. pylori*-IgG, *H. pylori*-IgM and *CMV*-IgG (O.R. =2.3, 1.9 and 1.7) respectively. Similarly non-alcoholic were found more seropositive for *C. pneumoniae*-IgA, *H. pylori*-IgA and hs-CRP group (O.R. =1.4, 1.2 and 1.6) respectively. Patients with family history of CAD and BP and Diabetes were more associated with *H. pylori*-IgG, *H. pylori*-IgM, *CMV* IgG and *C. pneumoniae*-IgA, *C. pneumoniae*-IgM, *H. pylori*-IgG, *CMV*-IgM and *C. pneumoniae*-IgA, *H. pylori*-IgM, *CMV*-IgM respectively.

Classification of IgA seropositivity in hsCRP and pro-inflammatory interleukin (IL-2, IL-6 and TNF-α) groups

In group with low Cp-IgA index value consisting of 37 CAD patients and 22 controls, positivity for hsCRP (16 Vs 6 respectively) was significantly higher ($p=0.001$) in CAD patients compared to controls, however, there was no significant difference for IL-6 (16 Vs 8 respectively), IL-2 (6 Vs 2 respectively) and TNF- α (4 Vs 1 respectively). In second group with medium IgA, index value (61 CAD patients and 36 controls) positivity for hsCRP (43 Vs 7 respectively) and IL-6 (35 Vs 10 respectively) was significantly higher ($p<0.001$ and 0.006) in CAD patients compared to controls, however differences were not significant for IL-2 (15 Vs 6 respectively)

and TNF- α (12 Vs 5 respectively). Similarly in third group with high IgA index value (57 CAD-pt and 18 controls), positivity for hsCRP (34 Vs 6 respectively) and IL-6 (32 Vs 4 respectively) was significantly higher (p<0.001, 0.011) in CAD patients compared to controls, however differences, were not significant for IL-2 (8 Vs 2 respectively) and TNF-α (6 Vs 3 respectively) (Table 3).

Table 3: Low, medium and high levels of *Chlamydia pneumoniae* specific IgA in hsCRP, IL-6, IL-2 and TNF-α group of coronary artery disease patients and controls

Pro-inflammatory markers	low IgA			medium IgA			high IgA		
	CAD-pt (N=37)	Control (N=22)	P value	CAD-pt (N=61)	Control (N=36)	P value	CAD-pt (N=57)	Control (N=18)	P value
hsCRP	16 (43.2)	06 (27.2)	0.001*	43 (70.5)	07 (19.4)	<0.001*	34 (59.6)	06 (33.3)	<0.001*
IL-6	16 (43.2)	08 (36.3)	0.405	35 (57.3)	10 (27.7)	0.006*	32 (56.1)	04 (22.2)	0.011*
IL-2	06 (16.2)	02 (9.1)	0.200	15 (24.6)	06 (16.6)	0.069	08 (14)	02 (11.1)	0.358
TNF-α	04 (10.8)	01 (4.5)	0.244	12 (19.6)	05 (13.8)	0.104	06 (10.5)	03 (16.6)	0.324

CAD-pt =coronary artery disease patients, hsCRP= high sensitive C-reactive protein, * = significant, percentage in parenthesis

Correlation analysis:

Positive and significant correlation (p<0.001, r = 0.424) was found between hsCRP and IL-6 in CAD patients (Fig. 3a). However, correlation was not significant between IL-6 and IL-2 in CAD patients (p=0.084, r = 0.158) (Fig. 3b).

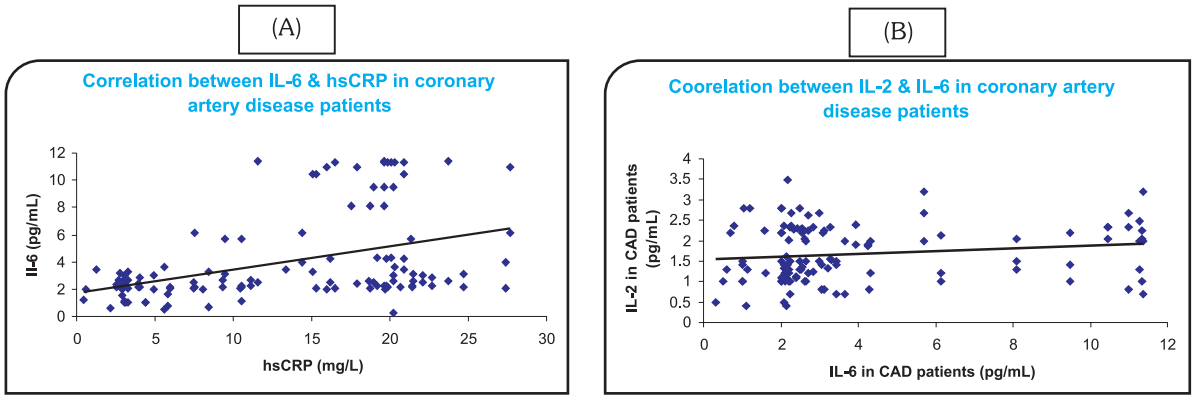


Fig. 3 A & B:CAD=coronary artery disease, *=significant, S.D. = standard deviation, IL=interleukin, ng=nano gram, pg=piko gram, mL=mili liter, IFN=interferon, ICAM= Intercellular adhesion molecule, VCAM= vascular adhesion molecule.

Evaluation of plasma circulatory markers in CAD patients and controls

Levels of plasma circulatory markers, viz.: IL-4, IL-8, IL-13, ICAM-1 and VCAM-1 were significantly higher ($p<0.001$) in contrast to levels of IL-10 and IFN- λ which were significantly lower ($p<0.001$) in CAD patients as compared to controls (Table 4).

Table 4: Evaluation of plasma circulatory markers in coronary artery disease patients and controls

Plasma circulatory markers	CAD patients (N=192)	Controls (N=192)	P value
	mean±S.D.	mean±S.D.	
IL-4 (pg/mL)	1.29 ±0.17	0.97±0.12	<0.001*
IL-8 (pg/mL)	3.27±0.31	2.36±0.22	<0.001*
IL-10 (pg/mL)	1.83±0.16	1.95±0.19	<0.001*
IL-13 (pg/mL)	4.81±0.28	4.25±0.27	0.004*
IFN-λ (pg/mL)	1.58±0.14	1.55±0.16	<0.001*
ICAM-1 (ng/mL)	14.38±1.16	13.12±1.11	<0.001*
VCAM-1 (ng/mL)	73.91±6.71	54.24±4.84	<0.001*

Evaluation of plasma circulatory markers in *C. pneumoniae* IgA positive CAD patients and controls

Mean levels of IL-4, IL-8, IL-13, ICAM-1 and VCAM-1 were significantly higher ($p<0.001$, <0.001, <0.001, <0.001 and <0.001) in *C. pneumoniae* IgA positive CAD patients as compared to *C. pneumoniae* IgA negative CAD patients, whereas IFN- λ was significantly lower ($p=0.033$). Mean levels of IL-4, IL-8, ICAM-1 and VCAM-1 were significantly higher ($p=0.007$, <0.001, 0.017, <0.001) in *C. pneumoniae* IgA positive controls as compared to *C. pneumoniae* IgA negative controls (Table 5).

Evaluation of plasma circulatory markers in hsCRP positive CAD patients and controls

Mean levels of plasma circulatory markers IL-4, IL-8, IL-13, ICAM-1 and VCAM-1 were significantly higher ($p=0.004$, <0.001, <0.001, <0.001, <0.001) in hsCRP positive CAD patients as compared to hsCRP negative CAD patients. Additionally, mean levels of IL-8 and VCAM-1 were significantly higher ($p<0.001$, <0.001) in hsCRP positive controls as compared to hsCRP negative controls (Table 6).

Table 5: Evaluation of plasma circulatory markers in *Chlamydia pneumoniae* IgA positive coronary artery disease patients and controls

Plasma circulatory markers	CAD patients		P value	Controls		P value
	IgA (+ve) (n=155)	IgA (-ve) (n=37)		IgA (+ve) (n=77)	IgA (-ve) (n=115)	
	mean± S.D.	mean± S.D.		mean± S.D.	mean± S.D.	
IL-4 (pg/mL)	1.31±0.13	1.17±0.12	0.007*	1.06±0.08	0.92±0.08	<0.001*
IL-8 (pg/mL)	3.41±0.31	2.76±0.26	0.015*	2.46±0.23	2.31±0.21	0.444
IL-10 (pg/mL)	1.82±0.11	1.86±0.11	<0.001*	1.95±0.14	1.96±0.15	0.879
IL-13 (pg/mL)	4.84±0.41	4.70±0.42	0.918	4.34±0.36	4.20±0.40	0.773
IFN- λ (pg/mL)	1.57±0.14	1.65±0.12	<0.001*	1.54±0.14	1.56±0.13	0.006*
ICAM-1 (ng/mL)	14.54±1.21	13.75±1.24	0.048*	13.16±1.10	13.10±1.09	0.528
VCAM-1 (ng/mL)	74.33±6.27	72.21±6.15	0.454	54.39±4.76	54.10±4.39	0.564

CAD=coronary artery disease, *=significant, S.D. = standard deviation, IL=interleukin, ng=nano gram, pg=piko gram, mL=mili liter, IFN=interferon, ICAM= Intercellular adhesion molecule, VCAM= vascular adhesion molecule, IgA=immunoglobulin A, +ve=positive, -ve=negative.

Table 6: Evaluation of plasma circulatory markers in high sensitive C - reactive protein positive coronary artery disease patients and controls

Plasma circulatory markers	CAD patients		P value	Controls		P value
	hsCRP (+ve) (n=116)	hsCRP (-ve) (n=76)		hsCRP (+ve) (n=60)	hsCRP (-ve) (n=132)	
	mean± S.D.	mean± S.D.		mean± S.D.	mean± S.D.	
IL-4 (pg/mL)	1.31±0.12	1.26±0.11	0.719	1.00±0.13	0.96±0.12	0.090
IL-8 (pg/mL)	3.32±0.31	3.20±0.26	0.573	2.53±0.21	2.29±0.18	0.207
IL-10 (pg/mL)	1.82±0.13	1.85±0.14	<0.001*	1.94±0.15	1.96±0.24	<0.001*
IL-13 (pg/mL)	4.86±0.30	4.74±0.31	0.118	4.30±0.33	4.23±0.30	0.668
IFN- λ (pg/mL)	1.57±0.12	1.59±0.13	<0.001*	1.54 ±0.19	1.56±0.17	<0.001*
ICAM-1 (ng/mL)	14.53±1.07	14.16±1.17	0.563	13.25±1.13	13.06±1.09	0.330
VCAM-1 (ng/mL)	74.20±7.21	73.47±6.91	0.658	54.60±4.92	54.10±4.39	0.874

CAD=coronary artery disease, *=significant, S.D. = standard deviation, IL=interleukin, ng=nano gram, pg=pico gram, mL=mili liter, IFN=interferon, ICAM= Intercellular adhesion molecule, VCAM= vascular adhesion molecule, hsCRP=high sensitive C –reactive protein, +ve=positive, -ve=negative.

6. ROLE OF IRON IN PATHOGENESIS OF *CHLAMYDIA TRACHOMATIS*

Scientific Staff	:	Harsh Vardhan, Dr. Aruna Singh
Duration	:	2006 - 2010

Aims, Objectives & Background

Chlamydia trachomatis (CT) is one of the most successful and leading bacterial pathogens that causes sexually transmitted infections and it infects and grows within the genital mucosal epithelial cells. Chlamydial infection is responsible for pelvic inflammatory disease, tubal infertility, ectopic pregnancy and endometritis, having significant impact on the reproductive health of women worldwide. The developmental cycle of *CT* includes two forms: an infectious elementary body (EB) and a reticulate body that multiplies within the inclusion by binary fission. A third developmental form - the persistent form, is considered as a mechanism of survival under stressful conditions. Persistence is induced in response to changes in the culture medium, including amino acid or iron deprivation and in the presence of antibiotics or cytokines such as gamma interferon (IFN-γ). Iron restriction induces persistence of chlamydial infection, simultaneously decreases infectivity as well as virulence, which will revert back when iron is added. Iron in turn plays a major role in macrophage interactions 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 upregulated in iron-depletion. In order to develop appropriate therapeutic regimen, it is essential to define the activation pathways wherein 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 postranscriptionally 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.

Work done during the period

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 *Chlamydia trachomatis* infected HeLa 229 cells at the interface of iron acquisition and its commensal utilization.

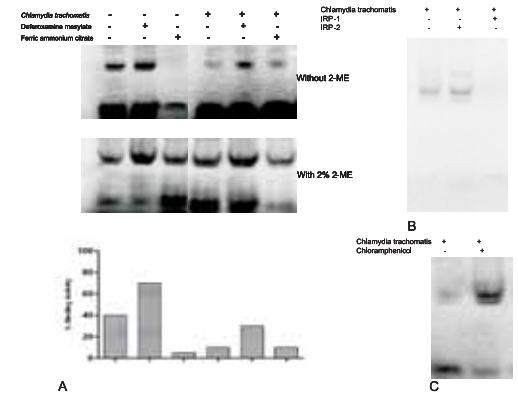


Fig 1

Fig 1: (A) Western blot showing 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.

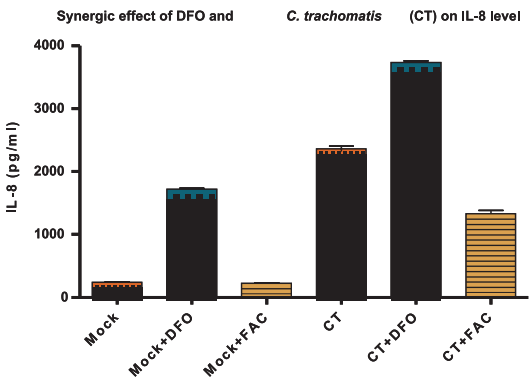


Fig 2

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

Scientific Staff	:	Rishein Gupta, Dr. Aruna Singh
Collaborators	:	Dr. Sudha Salhan, Safdarjung hospital
Technical Staff	:	Mrs. Madhu Badhwar, Mrs. Asha Rani
Duration	:	2006-2009

Aims, Objectives & Background

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 *C. trachomatis* (CT).

Work done during the period

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 (1β), 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-1β, 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 ($P<0.05$) (Fig. 2). Our overall data 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.

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

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]

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.

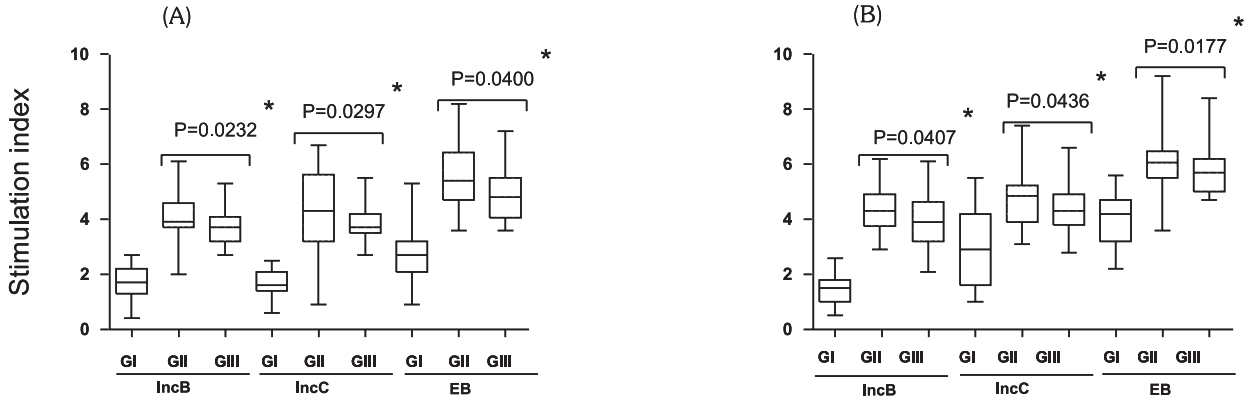


Fig. 1: MTT assay showing proliferative responses
Proliferative responses (stimulation indices) of (A) cervical cells and (B) PBMCs isolated from GI, GII, GIII on stimulation with IncB, IncC and CT EB were estimated by MTT assay.
(A) *Significant difference in proliferative responses in GII with respect 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.

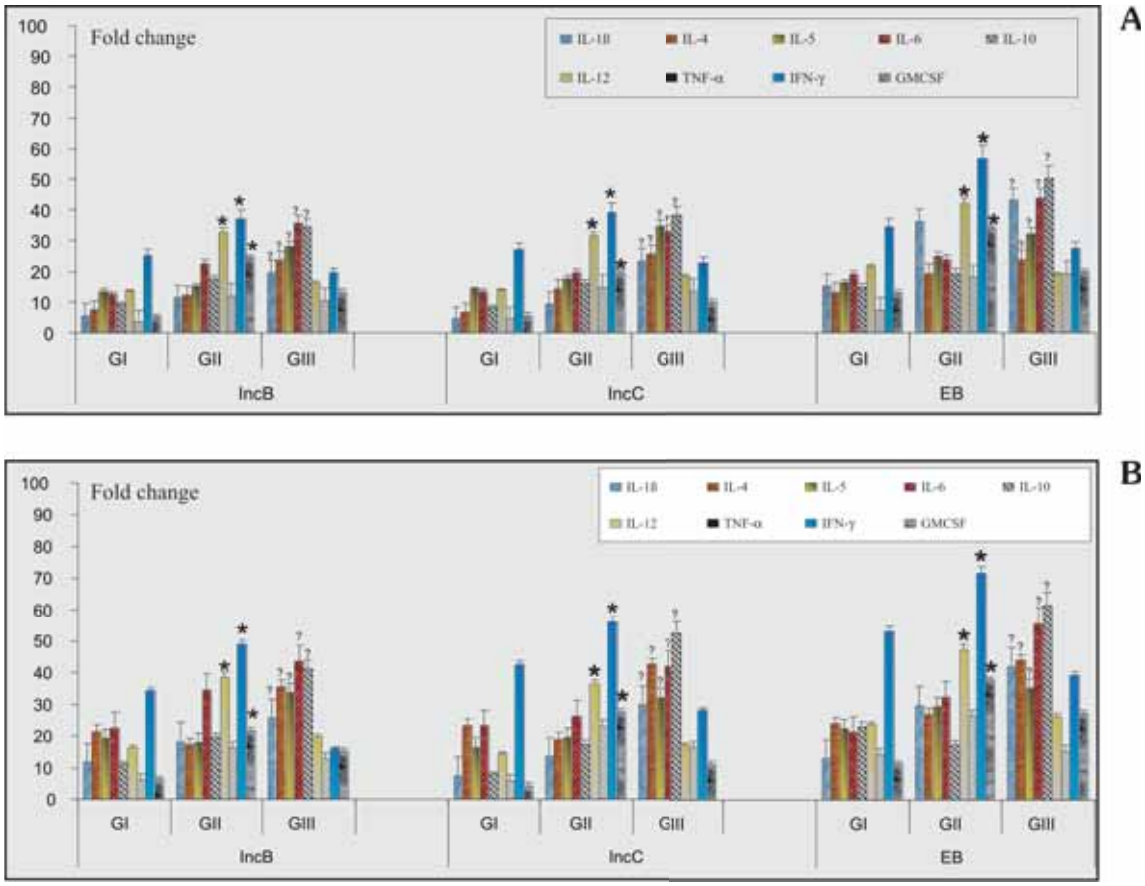


Fig. 2: Cytokine mRNA expression in Inc-stimulated cells

Estimation of mRNA expression of IL-1β, IL-4, IL-5, IL-6, IL-10, IL-12, TNF-α, IFN-γ and GMCSF in 0.6×10^5 (A) cervical cells and (B) peripheral blood mononuclear cells (PBMCs) upon *in vitro* stimulation with IncB, IncC and CT EB. Real-time RT-PCR analysis of mRNA levels was done at 12 hours post infection in cervical cells and PBMCs isolated from GI, GII and GIII.

* $P<0.05$ Expression of cytokine mRNA in GII compared to corresponding levels in GI and GIII by Kruskal Wallis test. $^{\Delta}$ $P<0.05$ Expression of cytokine mRNA in GIII compared to corresponding levels in GI and GII by Kruskal Wallis test.

8. ROLE OF OXIDATIVE STRESS IN *CHLAMYDIA TRACHOMATIS* INFECTED FIRST TRIMESTER SPONTANEOUS ABORTERS

Scientific Staff	:	Dr. Sangita Rastogi, Chanchal Yadav
Collaborators	:	Dr. Sudha Salhan, Dr. Banashree Das, Safdarjang hospital
Duration	:	2007-2010

Aims, Objectives & Background

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.

Work done during the period

During the period, 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. 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

Scientific Staff	:	Dr. Sangita Rastogi, Chanchal Yadav
Collaborators	:	Dr. Sudha Salhan, Dr. Banashree Das, Safdarjang hospital
Duration	:	2008-2011

Aims, Objectives & Background

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.

Work done during the period

This is an ICMR-funded SRF project. 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.

LEISHMANIASIS

1. DRUG RESISTANCE IN VISCERAL LEISHMANIASIS

Scientific Staff	:	Dr. Poonam Salotra, Dhiraj Kumar, Arpita Kulshrestha, Vasundhra Bhandari
Collaborators	:	Dr. N. S. Negi, Dr. V. Ramesh, Safdarjang hospital
Duration	:	2006-2011

Aims, Objectives and Background

Sodium antimony Gluconate (SAG) is the drug of choice against *Leishmania* and resistance to this drug is a major problem in the field not only in the Indian subcontinent, but also throughout the world. This increase in resistance to SAG has led to an upsurge in therapeutic failure, and with the limited chemotherapeutic alternatives, it is extremely relevant to know about the factors responsible for the resistance. In the previous year study, we had reported several genes which were upregulated in antimony resistant isolates identified using genomic microarray. Here, we report functional characterization of the selected genes.

Besides SAG other available drugs in India are Amphotericin B (AmB) as well as its liposomal formulation, Ambisome and oral drug Miltefosine (MIL). Both AmB and MIL are highly effective in antimony resistant patients but suffer from limitations of adverse reactions and cost. Moreover, anthroponotic VL transmission as well as long half-life and treatment duration pose threat of development of resistance to these drugs as well. Sitamaquine and paromomycin are the new antileishmanial drugs in the pipeline and are still under clinical trials. Our earlier studies with field isolates of *L.donovani* from SAG responsive and non-responsive patients revealed that the *in vitro* SAG susceptibility correlates with clinical response and resistance is prevalent in parasites from high endemicity (HR) zones. The studies designed here with Indian field isolates lead to understanding of natural susceptibility of Indian field isolates to antimony and other antileishmanial drugs, and the mechanism of Miltefosine resistance in field isolates.

Work done during the period

Transcriptome profiling for antimony resistance determinants in *Leishmania donovani* isolated from Indian patients of Kala-azar using genomic microarray helped us to identify the genes

that were uniquely expressed or showed altered expression in drug resistant strains, indicating their potential role in drug resistance. Genes coding for PSA-2, Histone 4 (H4), Histone 2A (H2A), Histone (H1), MAP-kinase, Nucleoside transporter and two hypothetical proteins were transcribed abundantly in the antimony resistant parasite in comparison to sensitive, while genes encoding amino acid transporter, Iron/Zinc 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 these genes was validated by semi-quantitative reverse transcriptase (RT)-PCR assay. For functional analysis, we selected 3 genes to evaluate their effect on the phenotype of *L.donovani* by over-expressing them in the SAG sensitive *L.donovani* isolates.

Generation of parasites over-expressing PSA-2, Histone H2A and Histone H4

The DNA encoding PSA-2/H2A/H4 was cloned in *L.donovani* expression vector pKSNeo and transfected into the two strains of *L.donovani* promastigotes (one WHO standard SAG sensitive strain and another SAG sensitive field isolate) by electroporation in 2mm gap cuvettes at 450 V, 500mF. Transfectants were selected for resistance to G418 (50µg/ml). Parasites transfected with the empty vector, pKSNeo, were used as mock-controls. Overexpression was confirmed by western blot using anti-HA monoclonal antibody (Fig. 1a). Further the lysates was probed with antibody against PSA-2 and Histone H2A to see the protein expression level before and after over-expression of the gene (Figs. 1b & 1c). Expression analysis was done with the help of the software "Image J" which showed the expression of PSA-2 to be almost 12 and 10 times higher in comparison to control in the K133 PSA-2^{++} and LdS PSA-2^{++}respectively (Fig. 1b) while in Histone H2A, it was 5 and 6 fold high respectively (Fig. 1c). The mutant parasites overexpressing PSA-2/Histone H2A/H4 were designated as LdS PSA-2^{++} / LdS H2A^{++}/K133 PSA-2^{++} /K133 H2A^{++}/LdS H4^{++} respectively.

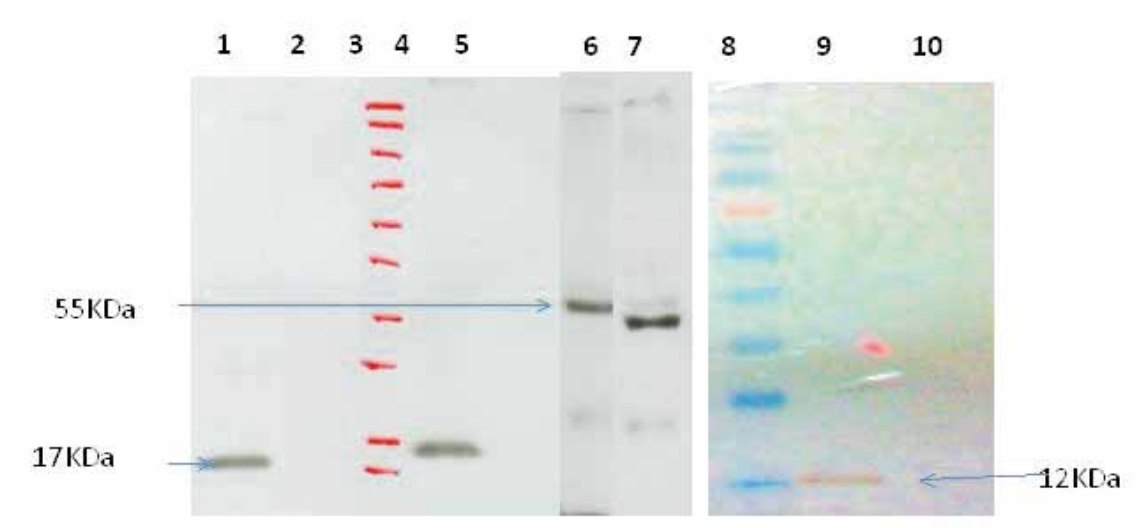


Fig.1a: Western blot analysis to confirm the presence of H2A (17KD), PSA-2(55KD) and H4 (12 KD) over expression construct in *Leishmania*. 100µg total promastigotes lysates were separated in a 12% SDS-PAGE gel and transferred to nitrocellulose membranes. Membrane was probed with anti HA antibody followed by HRP conjugated antibody and developed by using ECL.

Lane 1: LdS H2A^{++} Lane 2: LdS Neo
Lane 3: Marker Lane 4: K133 H2A^{++}
Lane 5: K133 Neo Lane 6: LdS PSA^{++}
Lane 7: K133 PSA^{++} Lane 8: Marker
Lane 9: LdS H4^{++} Lane 10: LdSNeo

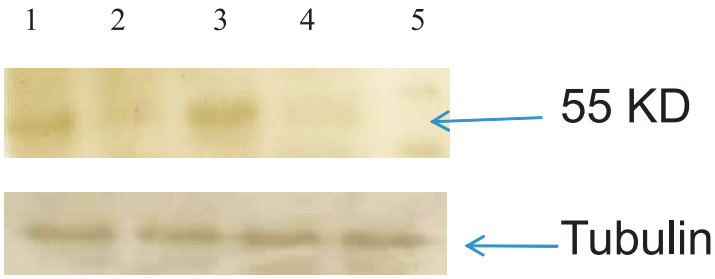


Fig. 1b: Western blot analysis for protein expression of PSA-2 (55KD) in *LdPSA-2*$^{++}$ compared with control. 100µg total promastigotes lysates were separated in a 12% SDS-PAGE gel and transferred to nitrocellulose membranes. Membrane was probed with antibody to PSA-2 followed by HRP conjugated antibody and developed by using ECL

Lane1 : K133PSA; Lane 2: K133N; Lane 3: Lds PSA;
Lane 4: Lds Neo; Lane 5 : marker

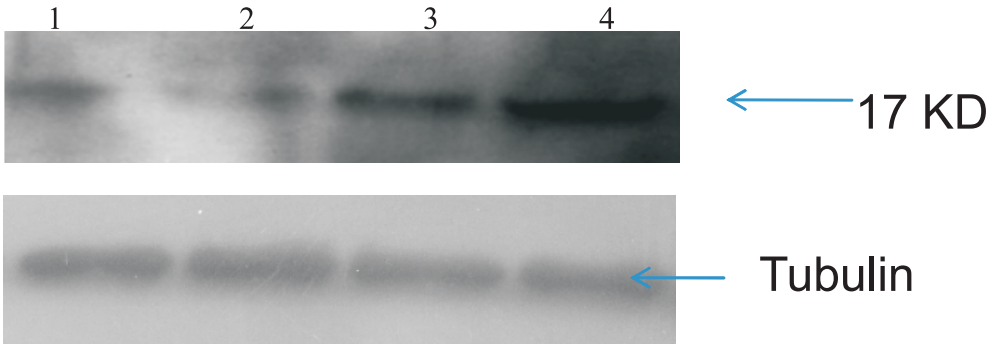


Fig. 1c: Western blot analysis for protein expression of Histone H2A (17 KD) in *LdH2A*$^{++}$ compared with control. 100μg total promastigotes lysates were separated in a 12% SDS-PAGE gel and transferred to nitrocellulose membranes. Membrane was probed with antibody to H2A followed by HRP conjugated antibody and developed by using ECL
Lane1 : K133PSA; Lane 2: Neo; Lane 3: Lds H_2A;
Lane 4: K133 HZA

Growth Kinetics of LdSPSA^{++} / LdSH2A^{++}/LdSH4^{++}

On day 0, *LdS Neo, LdS PSA-2*$^{++}$, *LdSH2A*$^{++}$, *LdS H4*$^{++}$, *K133 Neo, K133 PSA-2*$^{++}$, *K133 H2A*$^{++}$ parasites were diluted to 0.5 X10^5 cells/ml in 10 ml cultures to allow cells to enter logarithmic phase. For each time point, the parasites were counted by light microscopy using haemocytometer at 20X magnification. The growth pattern of all of the strains was found to be comparable with the control. (Fig. 2).

Drug Susceptibility of mutant parasites to SAG, Amphotericin-B and Miltefosine

An intracellular assay for *L. donovani* was performed using murine macrophage–adherent cell line J774A.1 in 8-well chamber slides. Cells were infected and incubated, for 48 h, with different concentrations of drug (SAG, MIL and AmB). After staining with Diff-Quik, number of *L. donovani* amastigotes per macrophage was microscopically counted at 100X magnification in 100 macrophages. The assay for each isolate was performed twice in duplicate. The percent killing was calculated by use of sigmoidal regression analysis (Origin 6.0; Origin Lab), and the ED_{50} was determined. *LdS H4*$^{++}$ showed similar Ed_{50} as compared to control, while *LdS PSA-2*$^{++}$, *LdS* H2A^{++}, *K133 PSA2-*$^{++}$*and K133 H2A*$^{++}$ showed significantly higher ED_{50} compared to control (Table 1) towards SAG (Fig. 3 A – B), AmB (Fig. 4 A – B) and MIL (Fig. 5 A – B).

Table 1: ED50 (μg/ml) of mutant parasites LdSPSA-2^{++}, LdSH2A^{++}, K133PSA2^{++}, K133H2A^{++} to SAG, Amphotericin-B and Miltefosine

Parasite ID	SAG	AmB	MIL
K133 Neo	6.28 ± 0.92	0.24 ± 0.007	1.4 ± 0.25
LdS Neo	6.37 ± 0.26	0.265 ± 0.007	1.46 ± 0.19
K133 H2A^{++}	79.31 ± 2.47	0.89 ± 0.007	2.91 ± 0.26
K133 PSA^{++}	70.19 ±3.56	1.11 ± 0.11	3.53 ± 0.14
LdS H2A^{++}	82.15 ± 7.12	0.96 ± 0.01	2.66 ± 0.45
LdS PSA^{++}	80.57 ± 5.01	1.08 ± 0.07	3.95 ± 0.11

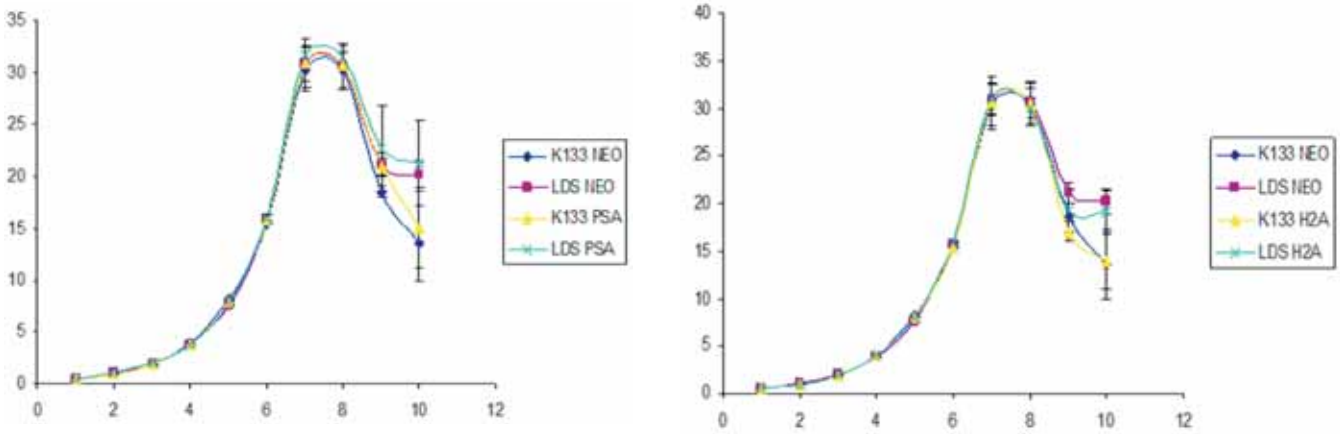


Fig. 2 (a): Growth curve of LdS PSA-2^{++}, K133 PSA-2^{++} in comparison with the control LdS Neo and K133Neo.
(b): Growth curve of K133H2A^{++} and *LdSH2A*$^{++}$ in comparison with the control K133 Neo, *Ld*SNeo.
The growth pattern was studies in triplicates and the value shown is the mean of the three values.

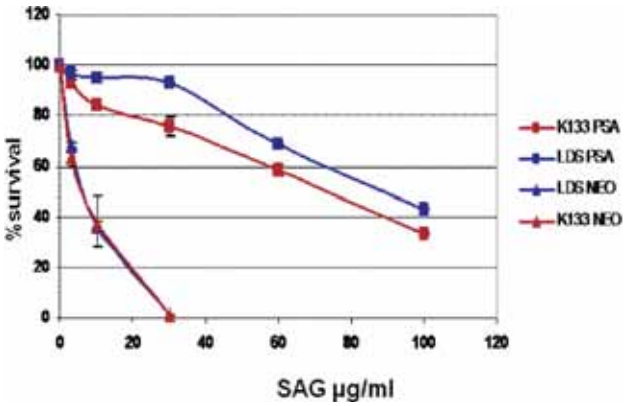


Fig. 3A: Susceptibility to SAG of intracellular *L. donovani LdSPSA-2*$^{++}$ and *K133PSA-2*$^{++}$ compared to control. Mean ED_{50} (µg/ml) of three separate experiments were plotted

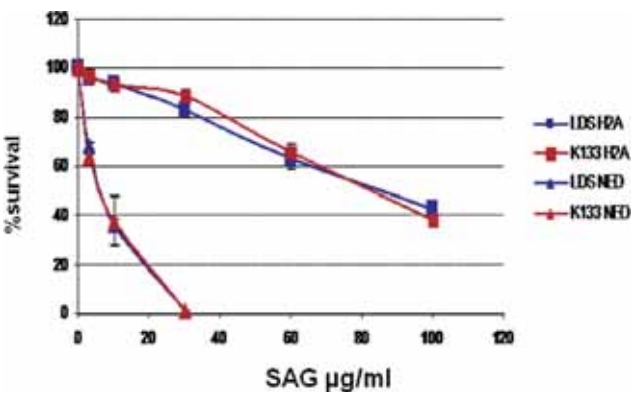


Fig. 3B: Susceptibility to SAG of intracellular *L. donovani LdSH2A*$^{++}$ and *K133H2A*$^{++}$ compared to control. Mean ED_{50} (µg/ml) of three separate experiments were plotted

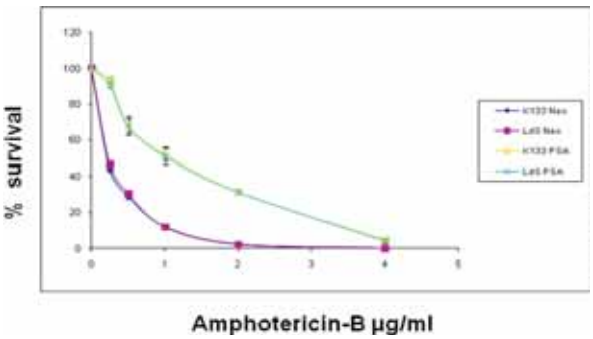


Fig.4A: Susceptibility to AmB of intracellular *L. donovani LdSPSA-2*$^{++}$ and *K133PSA-2*$^{++}$ compared to control. Mean ED_{50} (µg/ml) of three separate experiments were plotted

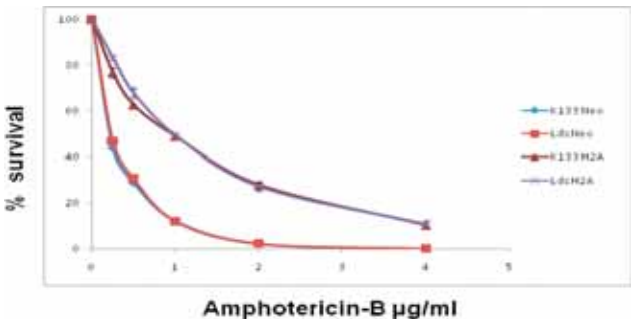


Fig. 4B: Susceptibility to AmBof intracellular *L. donovani LdSH2A*$^{++}$ and *K133H2A*$^{++}$ compared to control. Mean ED_{50} (µg/ml) of three separate experiments were plotted

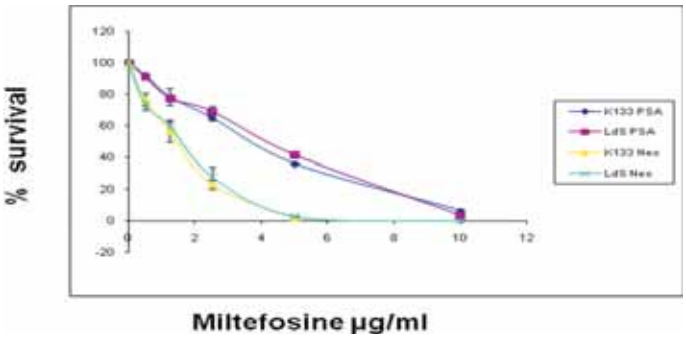


Fig. 5A: Susceptibility to MIL of intracellular *L. donovani LdSPSA-2*$^{++}$ and *K133PSA-2*$^{++}$ compared to control. Mean ED_{50} (µg/ml) of three separate experiments were plotted.

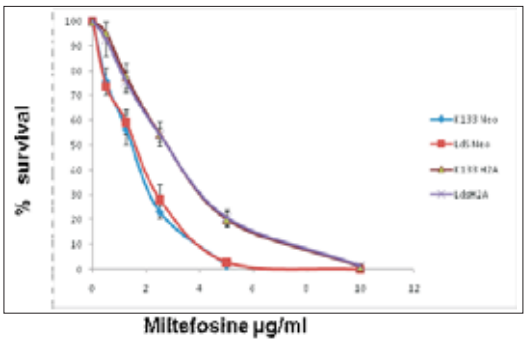


Fig. 5B: Susceptibility to MIL of intracellular *L. donovani LdSH2A*$^{++}$ and *K133H2A*$^{++}$ compared to control. Mean ED_{50} (µg/ml) of three separate experiments were plotted.

In vitro susceptibility of field isolates of *Leishmania donovani* to other antileishmanial drugs: correlation with SAG susceptibility and implications in field settings

We investigated anti-leishmanial activity of SAG, AmB and MIL in VL field isolates (n=19) from high and low endemicity regions in Bihar, India, which represent, the zones of low (LR) and high resistance (HR) to antimony, respectively. The isolates showed variable susceptibility to AmB (Fig 6) and MLF (Fig7). The ED_{50} at amastigote stage ranged from 0.17 to 0.77µg/ml for AmB and 0.48 to 2.32 µg/ml for MIL. We observed a strong correlation (r= 0.81) between MIL susceptibility of amastigotes and promastigotes (Fig. 7), while AmB susceptibility did not correlate significantly at amastigote and promastigote stage ($r = 0.32$, $p<0.05$) (Fig. 6). Isolates from HR region (n=12) had significantly higher mean ED_{50} ($p<0.01$) for all 3 drugs SAG, MIL and AmB as compared to LR region isolates (n=7). All isolates displayed significantly correlated susceptibility to the three drugs ($p<0.01$).

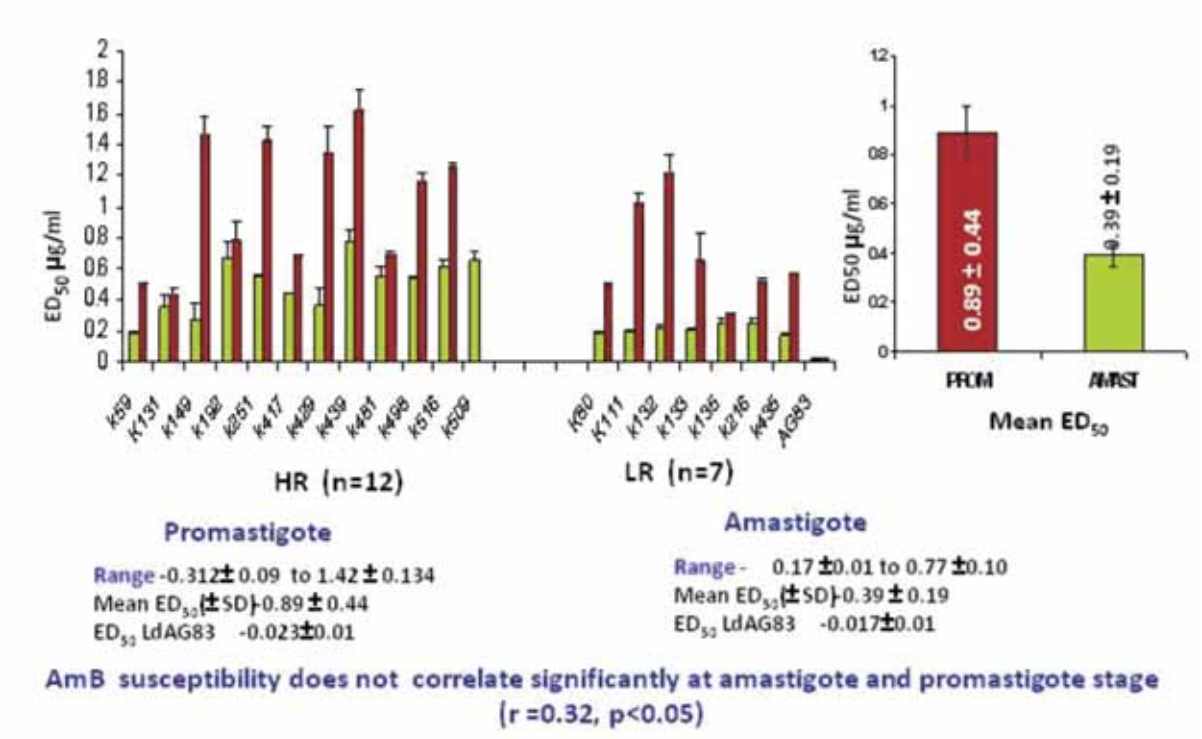


Fig. 6: *In vitro* Susceptibility of field isolates towards Amphotericin-B Green Bar Represents ED50 of Amastigotes while Red bar Represents ED50 of Promastigotes stage.

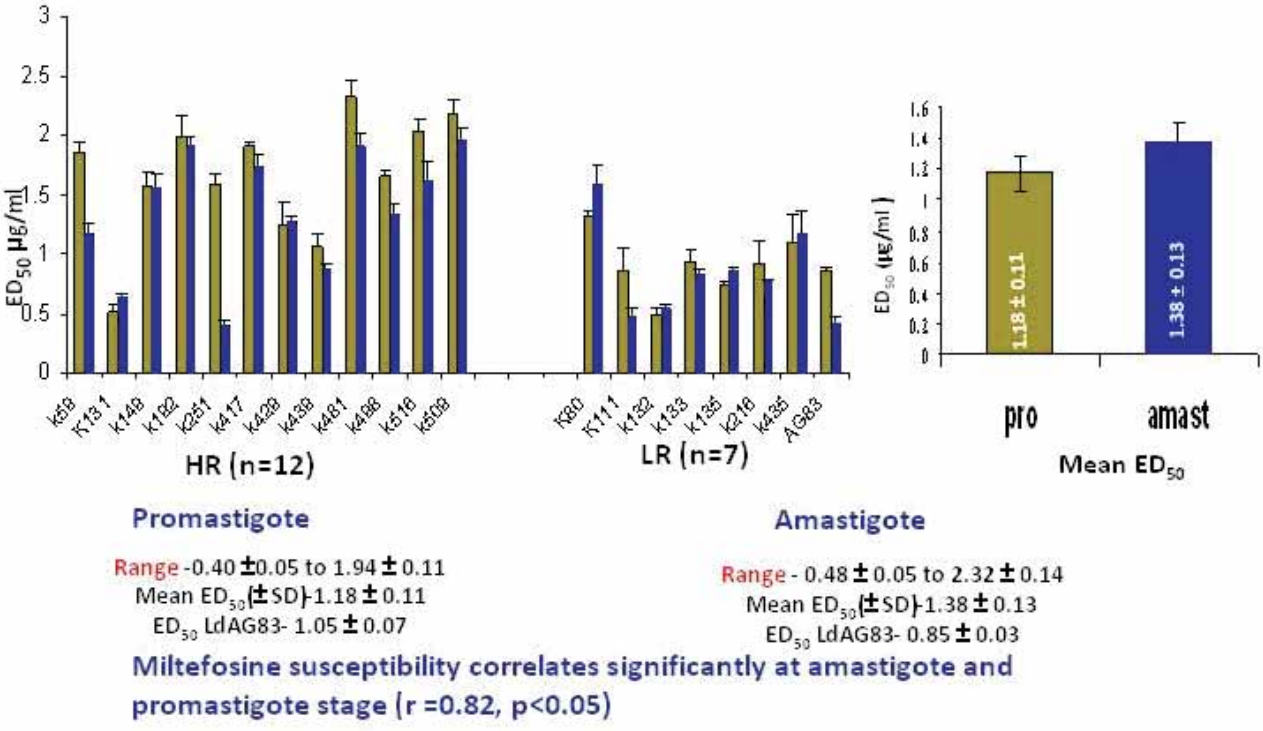


Fig. 7: *In vitro* Susceptibility of field isolates towards Miltefosine Green Bar Represents ED_{50} of Promastigotes while Blue bar Represents ED50 of Amastigotes stage.

We further extended the work to evaluate the susceptibility of clinical isolates to other available drugs 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±0.29μM for paromomycin and 0.57±0.07 to 3.74±0.78μM for sitamaquine (Fig. 8 A and B). The mean ED_{50} ± SEM for paromomycin and sitamaquine was 3.30±0.313μM and 2.15±0.209μM respectively. For sitamaquine, the isolates from HR region (n=13) had significantly higher mean ED_{50} ($p<0.05$) compared to LR (n=7) region parasites, a pattern seen for other antileishmanial drugs- SAG, AmB and miltefosine (Fig. 9).

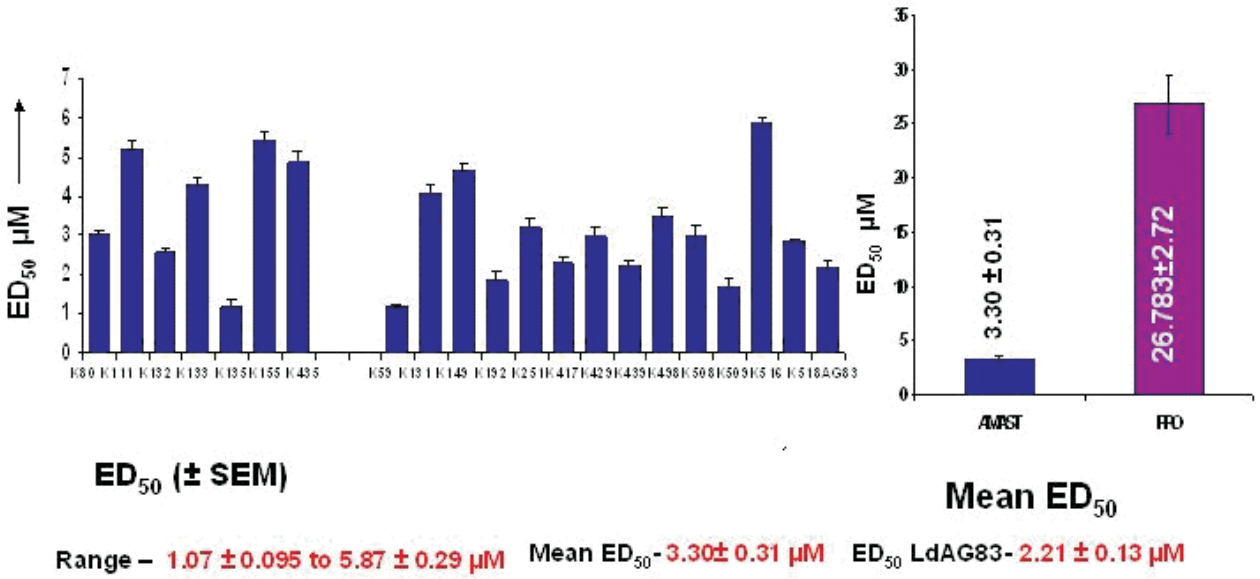
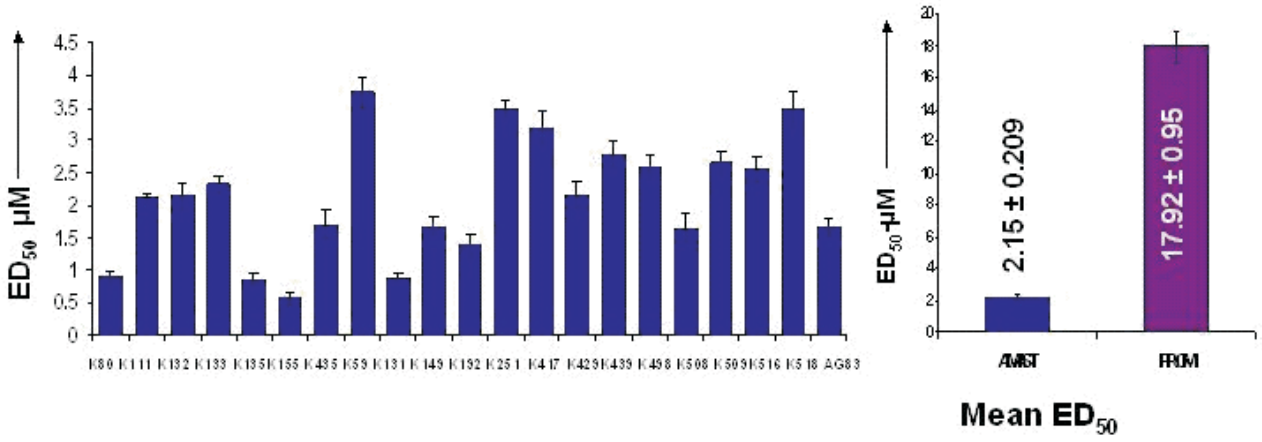
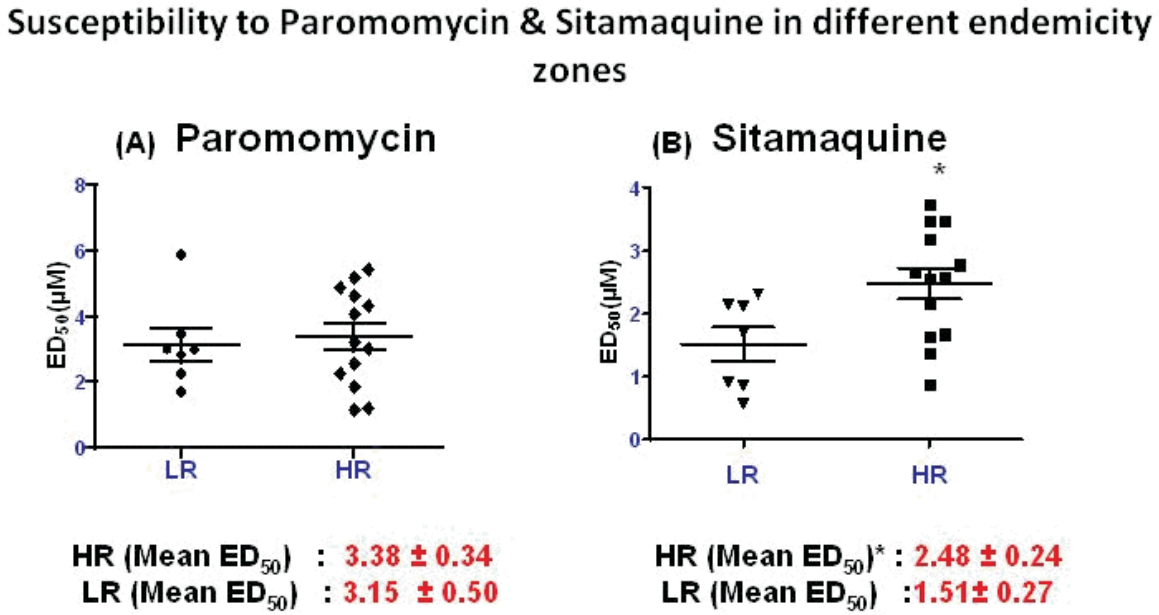


Fig. 8 A: *In vitro* Susceptibility of *L donovani* amastigotes towards Paromomycin



ED_{50} Range –0.57 ±0.08 to 3.74 ±28 µM Mean ED_{50}- 2.15 ± 0.209 ED_{50} LdAG83- 1.66 ± 0.14

Fig. 8B: *In vitro* Susceptibility of *L donovani* amastigotes towards Sitamaquine



HR (Mean ED_{50}) : 3.38 ± 0.34
LR (Mean ED_{50}) : 3.15 ± 0.50

HR (Mean ED_{50})* : 2.48 ± 0.24
LR (Mean ED_{50}) :1.51± 0.27

Fig 9: *In vitro* susceptibility of HR & LR region isolates towards Paromomycin (A) and Sitamaquine (B) Paromomycin had a comparable ED50 (µM) in both the endemicity areas. HR isolates had significantly higher mean ED50 ($p<0.05$) for sitamaquine than LR

Expression analysis of reported markers for miltefosine resistance in clinical isolates of *L. donovani*

In a series of studies with lab generated Miltefosine resistant parasites, MIL uptake is shown to correlate extraordinarily well with the sensitivity to the drug. The uptake levels and, therefore, the sensitivity depend on the expression levels of the functional flippase machinery at the plasma membrane constituted by transporter LdMT and its subunit LdRos3. To evaluate if similar mechanisms operate in field isolates, the gene expression of LdMT and LdRos3 was evaluated in VL field isolates (n=17) in comparison with the expression in *Ld*AG83 standard *L. donovani* strain and MIL resistant parasite *Ld*M20. Seven field isolates exhibited comparable expression of LdMT and 2 of these isolates had comparable expression of LdRos3 which was downregulated by more than 2-fold in the remaining isolates in comparison to *Ld*AG83 (range 1 to 8 fold less in case of LdMT and 1.6 to 83.33 fold less in case of LdRos3 (Fig. 10). In comparison to *Ld*AG83, majority of the isolates, except one (K192), revealed decreased expression of LdMT and LdRos3. The expression of LdMT was > 4 fold higher while the expression of LdRos3 was downregulated by ~ 2 fold in K192, suggestive of other mechanisms operating in the MIL activity. In general, expression of LdMT and LdRos3 was correlated and expression of LdRos3 was higher than LdMT, though the ratio of expression of LdMT and LdRos3 varied greatly among isolates (from 0.67 to 10 fold). Both labs adapted *Ld*M20 isolates exhibited decreased expression of LdMT and LdRos3. In comparison to the two lab generated MIL resistant parasite *Ld*M20, the expression of LdMT and LdRos3 was more than 3-fold in a majority of the isolates (14/17, 82.3%).

This work was published in Antimicrobial Agents and Chemotherapy, 53:835-8. 2009.

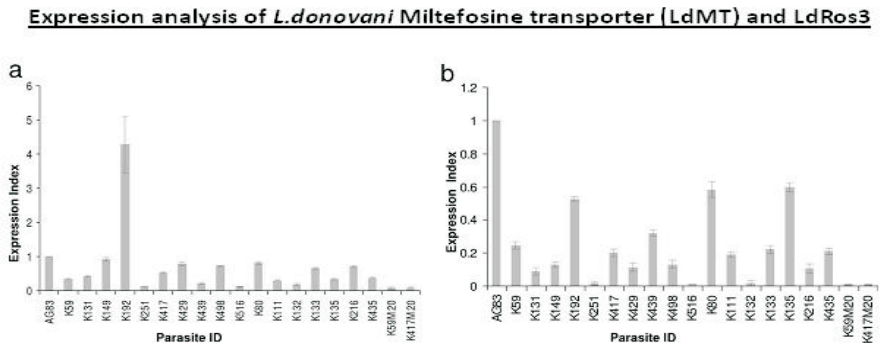


Fig. 10: Expression of LdMT and LdRos3 in different field isolates. (a) Real-time PCR expression analysis of the *L. donovani* MIL transporter (LdMT). The graph shows the expression index, defined as ratios of gene expression relative to that of strain *Ld*AG83.
(b) Real-time RT-PCR expression analysis of the *L. donovani* MIL transporter (LdRos3). The graph shows the expression index, defined as ratios of gene expression relative to that of strain *Ld*AG83. Data represent the means of the results of three independent experiments.

Future work

Transcriptome analysis for identification of genes upregulated in miltefosine resistance will be taken up. Mechanism of parasite killing by paromomycin and sitamquine will be investigated.

2. STUDIES ON CHARACTERIZATION OF E1-LIKE UBIQUITIN-FOLD MODIFIER ACTIVATING ENZYME (LDUBA5)

Scientific Staff	:	Dr. Poonam Salotra, Paresh Sharma, G Srividya
Duration	:	2008-2011

Aims, Objectives and Background

Studies on stage regulated gene expression in *Leishmania* are important since gene products that regulate differentiation of promastigotes into the intracellular amastigote form have great potential as targets to block the infection process. The microarray technology allowed us to identify a set of genes that were coordinately expressed during the process of differentiation from promastigote to amastigote differentiation. Expression pattern of selected genes were validated using Northern analysis, RT-PCR or quantitative real-time PCR experiments using total RNA from different stages of *Leishmania*. Since, a sufficient number (57) of differentially expressed clones were identified using 4224 clones chip, further hybridizations with ~10,000 chip was not considered necessary. Four genes that showed differential expression at various *Leishmania* life stages were chosen for cloning, expression and further characterization. These genes were a trypanosomatid specific gene (p27), E1-like ubiquitin-fold modifier activating enzyme (*Ld* UBA5), argininosuccinate synthase (ASS), calpain-like cysteine proteinase (CCP). Out of which p27 and ASS were characterized last year, while this year LdUba5 was chosen for further functional analysis.

Ubiquitin (Ub) and ubiquitin-like protein-conjugating enzymes play central roles in post-translational modification processes. The ubiquitin-fold modifier 1 (Ufm1), one of a variety of ubiquitin-like modifiers, covalently attaches to target proteins via Uba5 and Ufm1-conjugating enzyme 1 (Ufc1), which are analogous to the E1 and E2 ubiquitylation enzymes, reported in humans. Ufm1-related proteins are conserved in metazoa and plants but not in yeast.

While the majority of ubiquitin and ubiquitin-like (UBL) post-translational modifiers pathway in mammalian and yeast cells have been studied and characterized, relatively little is known about how these systems are used by parasites. The complex life cycles and multiple disease-causing states of parasitic protozoa offer a unique context in which to study ubiquitin and Ubl modification pathways. The life cycles of most protozoan parasites within single or multiple hosts rely on strict timing of protein regulation and gene expression for both survival and virulence. While the regulation of gene expression and protein turnover is clearly critical for both life cycle and disease progression in medically important protozoa, the mechanisms regulating these processes are not well understood. Given the known functions of ubiquitin and Ubls in other organisms, a better understanding of these posttranslational modifiers is likely to be critical to understand how parasites control many basic biological processes. UBLs (for example SUMO, RUB1/NEDD8, APG12) do not seem to promote proteasomal degradation, but regulate a variety of cellular functions as critical regulators of many cellular processes, such as transcription, DNA repair, signal transduction, autophagy and cell cycle.

Work done during the period

During the period under report, clone 29C8 encoding E1-like ubiquitin-fold modifier activating enzyme (LdUba5) that was found to be upregulated at the amastigote stage using *L. donovani* genomic microarray (Promastigote vs Axenic Amastigote) was selected for further analysis and characterization.

Detection of *Ld*Uba5 transcripts in amastigote stage and in bone marrow of VL patients

Stage regulated transcriptome profiling using *L.donovani* genomic microarray led to the identification of a genomic clone corresponding to *Ld* Uba5, representing a transcribed sequence that exhibited 3.49 (±0.24) fold higher expression at axenic Am stage. Upregulation of *Ld*Uba5 was validated by Real Time PCR in both axenic and hamster derived amastigotes, further *Ld*Uba5 gene transcripts were also shown in bone marrow samples from VL patients (Fig. 11). Since, *Leishmania* burden in different bone marrow samples directly correlated with the expression of *Leishmania* specific α-tubulin, the relative expression of various gene transcripts were determined with respect to tubulin expression. A1 gene overexpressed in the amastigote stage of *Leishmania* was included as a positive control. Initially, primer efficiency of all the primer pairs were determined using absolute quantification method and slope of each primer pair was observed to be ≥ -3. HPRT gene which was used as an endogenous

control was positive in all 4 bone marrow samples, however the transcripts of α-tubulin, A1, *Ld*Uba5 were not detectable in VL negative bone marrow.

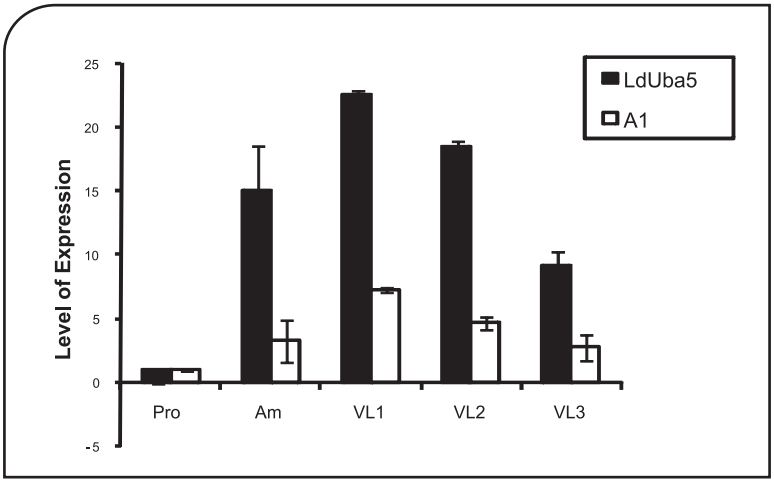


Fig. 11: The expression of *Ld*Uba5 gene in parasites and lesion tissues of VL patients. The expression of *Ld*Uba5 in axenic amastigotes (Am) with respect to promastigotes (Pro) using α-tubulin as endogenous controls. *Ld*Uba5 expression relative to Leishmania specific α-tubulin was determined in bone marrow derived RNA samples.VL1, VL2, VL3 correspond to samples from three confirmed cases of VL. Bar represents standard error from three experiments.

Sequence analysis, Cloning and Expression of rL*d*Uba5 in *L.donovani*

The *Ld*Uba5 sequence aligned within the ORF region of the gene LinjF15.1020 (NAD/FAD dependent dehydrogenase) in the *L.infantum* genome, which codes for a protein of ~43.2kDa. Primers were designed for ORF region of this *L.infantum* gene and amplified with the *L. donovani* DNA and sequenced. The *L.donovani* sequence showed 100% homology with the gene from *L.infantum*. Homology search of the open reading frame of *Ld*Uba5 revealed its similarity with a 404-amino-acid protein (Uba5), highly conserved in various multicellular organisms, including *Homo sapiens*, *Caenorhabditis elegans*, *Drosophila melanogaster*, and *Arabidopsis thaliana*, but absent in yeasts (*Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*) and a member of the E1-like enzyme family (Fig. 12 A). We named this protein *Ld*Uba5, based on its homology to human Uba5. Clustal W analysis of *Ld*Uba5 showed conserved cysteine residue similar to human Uba5 in *Leishmania* and *Tyrpanosoma*. *Ld*Uba5 also has an ATP-binding motif (GXGXXG) and a metal-binding motif conserved in other E1-

like enzymes such as Uba1, Uba2, Uba3, Uba4, and Atg7. Most of E1-like enzymes have an active site Cys residue within the conserved 10–20 amino-acid (AA) residues downstream from the metal-binding motif (Komatsu et al, 2004). For the production of recombinant *Ld*Uba5 protein, the gene was amplified using DNA isolated from *L.donovani* and cloned into pCR-T7 CT TOPO TA expression vector. A sufficient quantity of the recombinant *Ld*Uba5 protein was produced to inject into a rabbit for the production of antibodies to *Ld*Uba5.

Expression of *Ld*Uba5 at protein level in Pro and Ama stages of *L.donovani*

The antibodies raised against recombinant *Ld*Uba5 bind a single band of approximately ~ 45 kDa. Immunoblotting with the *Ld*Uba5 Ab revealed that this protein is expressed equally in both Pro as well as Ama stages of *L.donovani* (Fig. 12 B). Normalization of parasite lysates was carried out using anti-α-tubulin antibody. The expression at protein level was tested in two isolates of *L. donovani* and in two biological preparations each.

Cysteine^{217} is the active site residue of *Ld*Uba5

If an active site Cys residue within an E1 or E1-like enzymes is changed to Ser, an O-ester bond instead of a thioester bond is formed with its respective modifier protein and the intermediates become stable even under reducing conditions (Komatsu et al, 2004). In human Uba5, Cys^{250} is the active site residue, multiple alignment of *Ld*Uba5 along with human Uba5 showed Cys^{217} to be the most possible active site residue in *Ld*Uba5 (Fig. 12 A). Therefore, we mutated Cys within *Ld*Uba5 to Ser or Ala by site directed mutagenesis and prepared constructs Δ*Ld*Uba5^{C217S}or Δ*Ld*Uba5^{C217A} (Fig. 13 A). WT*Ld*Uba5 and mutated plasmid constructs were transfected into *L.donovani* promastigotes. Cell lysates were prepared from each of the transfected parasites at mid-log stage and analyzed by western blot using anti-*Ld*Uba5 Ab, subsequent to confirmation of transfection with anti-HA antibodies (Fig. 13 B). Parasites transfected with *Ld*Uba5 or Δ*Ld*Uba5^{C217A} gave a single band of ~45kDa (Fig. 13 B, lane 2, 4) while those transfected with Δ*Ld*Uba5^{C217S} showed an additional band of ~57kDa in addition to 45kDa, indicating that Δ*Ld*Uba5^{C217S} forms an intermediate complex with an endogenous protein (Fig. 13 B, lane 3). Cell lysates from wild type (WT) *Leishmania* cells used as control showed a band of ~45kDa with anti-*Ld*Uba5 Ab (Fig. 13 B, lane 1).

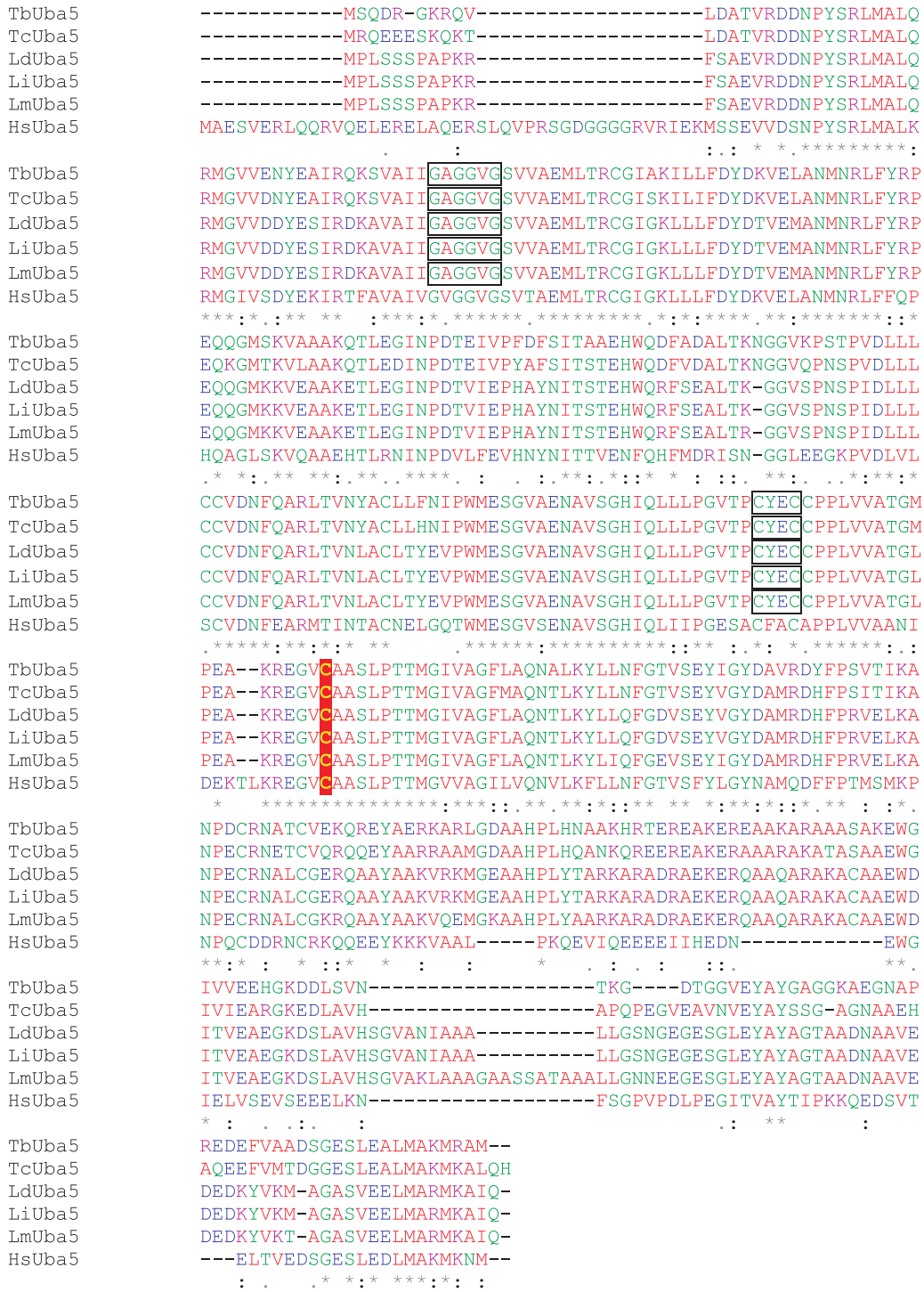


Fig. 12: A Multiple alignment of various Uba5 sequences :Hs, *H.sapiens* (AK026904); *Ld*, *L.donovani*(); Lm, *L.major* (LmjF15.0970); Tb,*T.brucei* (Tb09.160.4430); Tc, *T.cruzi* (Tc00.1047053505843.40). The accession numbers for the *Leishmania* and *Trypanosoma* genes are available from GeneDB while the Human Uba5 is available from GenBankK data bank. The amino-acid sequence of Uba5 is compared by Clustal W program. Asterisks denote identical amino acids; single and double dots denote weakly and strongly similar amino acids, respectively, determined by the criteria of ClustalW program. Amino acids are listed in the standard one letter code, and residues identical to Human Uba5 are indicated by dark boxes. The putative active site Cys residue is boxed in red. Open box indicates an ATP-binding motif (GXGXXG). The metal binding motif (CYEC) is underlined.

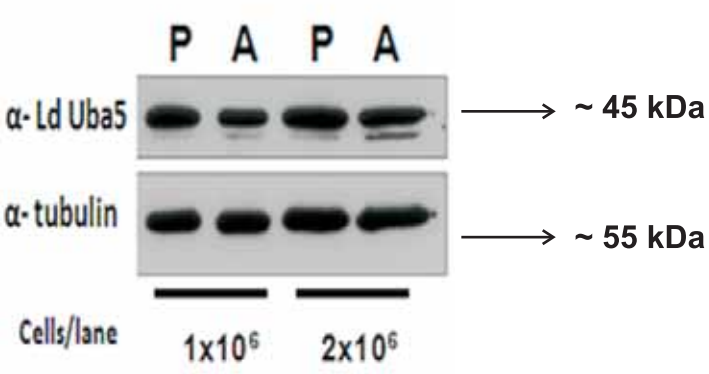


Fig.12B: Western blot analysis using LdUba5 antibodies. Cell lysates (25μg of protein) from promastigote (P) as well as amastigote (A) stages were run on a 12% SDS-PAGE and analyzed by Western blot. The blots were probed with anti LdUba5 using α tubulin Ab for normalization. Molecular sizes of the bands are indicated.

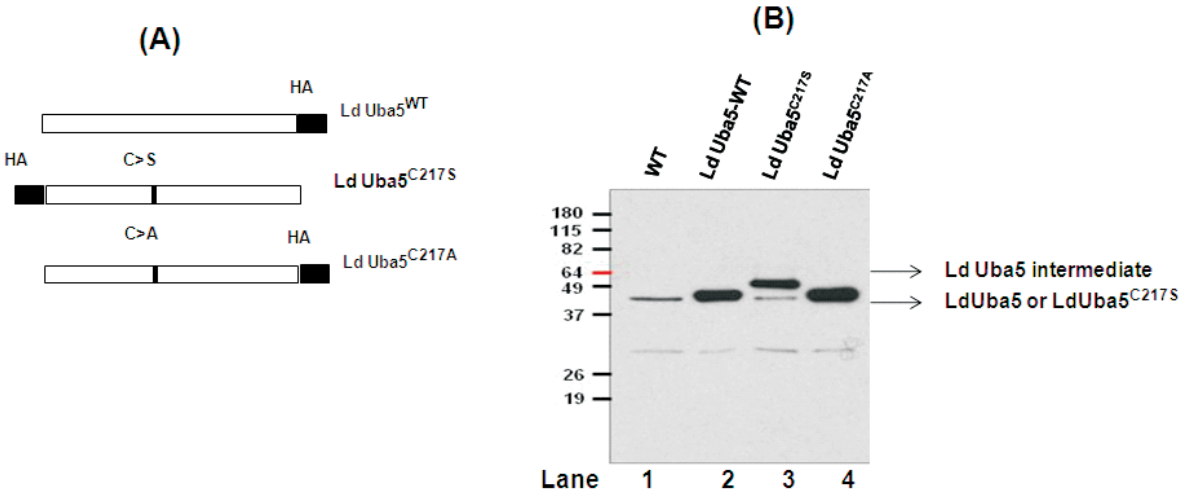


Fig.13: Identification of the intermediate linked to *Ld*Uba5 in *Leishmania* cells.
(A) Schematic representation of *Leishmania* expression plasmids for *Ld*Uba5 and the derivative mutants. *Ld*Uba5 was constructed with HA tagged at the C-terminus (*Ld*Uba5^{WT}). To construct *Ld*Uba5^{C217S} and *Ld*Uba5^{C217A}, Cys^{217} of HA-*Ld*Ufm1 was mutated to Ser/Ala by site-directed mutagenesis. The *Ld*Uba5^{C217S} and *Ld*Uba5^{C217A} mutants were tagged with HA epitope at the N-terminus and C-terminus respectively.
(B) Cell lysates (25μg) from parasites transfected with *Ld*Uba5 (WT), Δ*Ld*Uba5^{C217S}(C>S) or Δ*Ld*Uba5^{C217A}(C>A) were subjected to SDS–PAGE and analyzed by immunoblotting with α-*Ld*Uba5 purified IgG. Cell lysate from wild type *L.donovani* cells were used as control.

*Ld*Uba5 mutants have a dominant negative effect on the growth of the parasite

As a first step towards understanding the role of *Ld*Uba5 growth of parasites was analyzed in culture. The *Ld*Uba5++ parasites showed growth comparable with the control cells. On the other hand, parasites expressing Δ*Ld*Uba5^{C217S}++ or ^{C217A}++ displayed a marked (10-fold) reduction in the growth rate compared with the controls (Fig14).

Sub cellular localization of *Ld*Uba5 in *Leishmania* cells

To determine the cellular localization of *Ld*Uba5 in *Leishmania*, antibodies against r*Ld* Uba5 were used in immunofluorescence which revealed that *Ld*Uba5 is localized in mitochondria in promastigotes as well as axenic amastigotes of *L.donovani*, as indicated by its colocalization with Mitotracker Red, a mitochondrial marker (Fig.15). Rabbit non-reactive serum (NRS) was used as control.

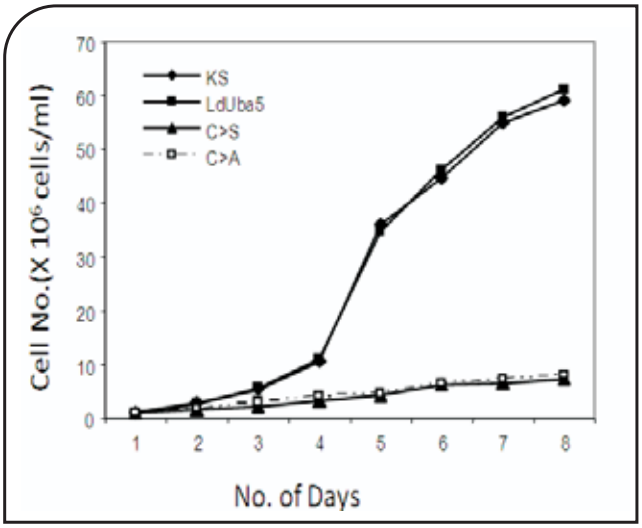


Fig.14: Effect of the expression of *Ld*Uba5 and *Ld*Ufm1 and their mutants on the growth of the parasite. (A) Growth of parasites transfected with either *Ld*Uba5 or vector (KS). (B) Growth of the parasites transfected with either *Ld*Uba5 or mutant *Ld*Uba5(C>S and C>A) and vector (KS).

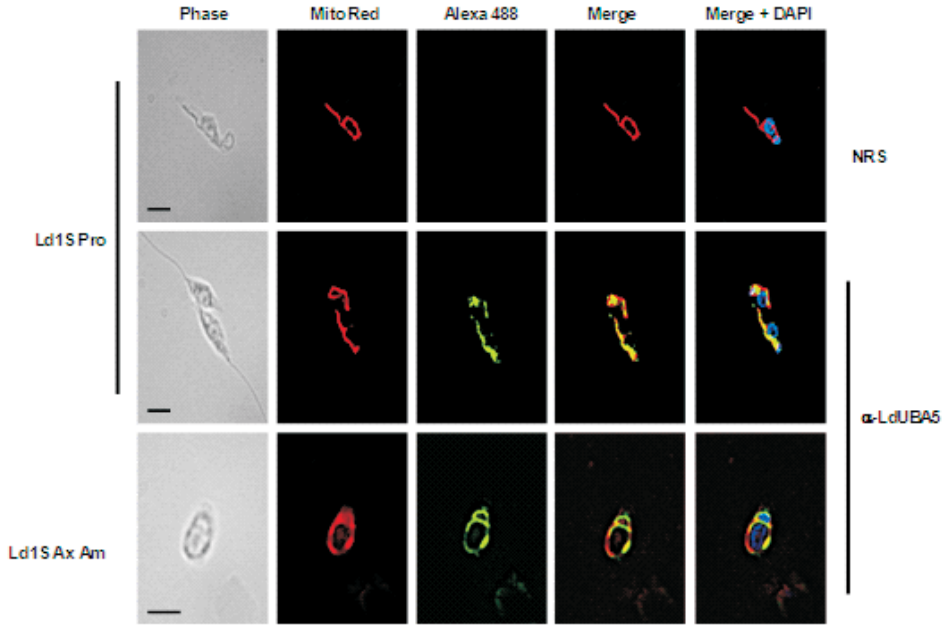


Fig.15: Immunolocalization of *Ld*Uba5 in promastigotes and axenic amastigotes. Immunofluorescence analysis of both promastigotes (pro) and axenic amastigotes (Am) using rabbit anti-*Ld*Uba5 Ab (Images1 and 2). Nucleus (N) and kinetoplast (K) were stained with DAPI (Images 3 and 4). Images were viewed under the confocal microscope. The stained images and phase were merged and shown (Images 5 and 6).

Future work

To dissect the *L. donovani* specific pathway involving LdUba5 , we propose to identify other components of the pathway as desribed in the human system. Specifically, we will investigate for presence of an Ubiquitin-like modifier 1 (Ufm1) and E2-like conjugating enzyme (Ufc1) in *L. donovani*. Further, the biological role of the *Ld*Ufm1-modifying system will be assessed in parasites.

3. ANALYSIS OF HOST IMMUNO-DETERMINANTS INVOLVED IN PATHOGENESIS OF KALA-AZAR AND POST-KALA-AZAR DERMAL LEISHMANIASIS USING cDNA ARRAY

Scientific Staff	:	Dr. Poonam Salotra, Dr. Nasim Akhtar Ansari, Gajendra Kumar Katara
Collaborators	:	Dr. V. Ramesh, Dr Sumita Saluja, Safdarjang hospital
Duration	:	2006 – 2009

Aims, Objectives and Background

Previously, we have documented significant levels of Interferon (IFN)-γ, Tumour Necrosis Factor (TNF)-α, Interleukin (IL)-10, Transforming growth factor (TGF)-β and IL-6 in localized lesion tissues of PKDL patients and TNF-α as well as Nitric Oxide (NO) in serum samples. In spite of the presence of effector molecules like IFN-γ, TNF-α and NO during active disease, the parasite persists, implying that the biological processes involved in the disease pathogenesis are complex and cannot be interpreted as simple Th1 or Th2-mediated processes, characteristic of the murine model of leishmaniasis. Recent reports suggested the possible role of impaired IFN-γ receptor induced signaling in *Leishmania* infection which may be responsible in reducing the effect of high IFN-γ levels in the disease.

Many other genes aside cytokines are considered to be responsible for determination of severity and the progression of the disease. Results of our recent study reflect involvement of other molecules like TNF-receptors (TNFR), matrix metalloproteinases (MMPs) and tissue inhibitors of matrix metalloproteinases (TIMPs), suspected as immuno-determinants in *Leishmania* pathogenesis. Among these TNFR1 were found modulated in both PKDL and KA. Most of the studies for understanding *Leishmania* pathogenesis have been performed using human cell lines and mouse models. The present study was designed to understand the host immune responses in localized tissue BMA of KA and dermal lesion tissue of PKDL patients. This study was carried out under a project funded by LSRB, DRDO.

Work done during the period

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 PKDL and 10 KA patients. The data provided evidence for both inflammatory and non-inflammatory responses, as reflected by elevated tumour necrosis factor (TNF)-α and interleukin (IL)-10 in PKDL lesions compared with normal skin tissue (n = 6) (Fig. 16). The ratio of TNF-α: IL-10 message was 2·66 in PKDL cases, substantially higher than in KA (1·18). Investigation of TNF-α receptors (TNFR1 and TNFR2) revealed a significant down-regulation of TNFR1 transcript in both PKDL and KA compared with control (Fig. 17). In the presence of elevated levels of TNF-α transcript, interference with type 1 effector activity in PKDL may be due to minimal expression of the TNFR1 gene. Investigation of (MMPs), known to be induced by TNF-α, and the (TIMPs), provided evidence for the roles of TIMP-1 and TIMP-3 in the pathogenesis of PKDL (Fig. 18).

This work was published in Clin Exp Immunol. 154:391-8, 2008.

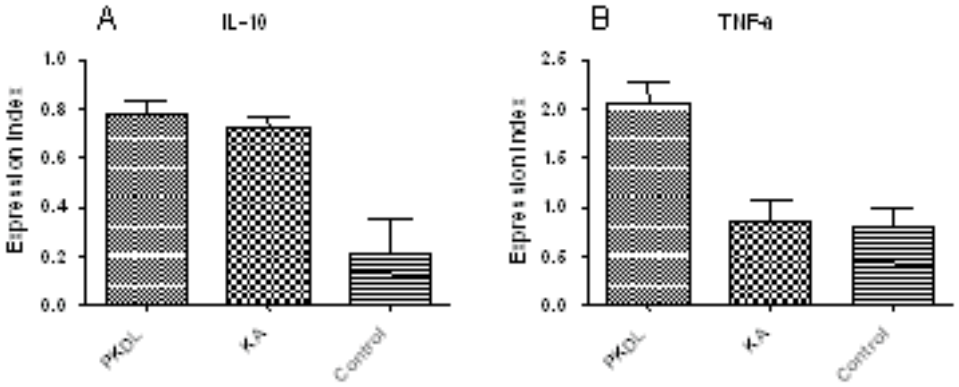


Fig. 16: Transcripts of Interleukin-10 [IL-10] (A) and Tumor necrosis factor-α [TNF-α] (B) in lesion tissues from patients with PKDL (n=14) and KA (n=10) and control tissues (n=6). Normalized cDNA was amplified with respective cytokine primers.. The graph shows results as an expression index, defined as the ratio of the intensity of cytokine with respect to the HPRT gene. The bars indicate SEs. $P < 0.05$ was considered to be statistically significant.

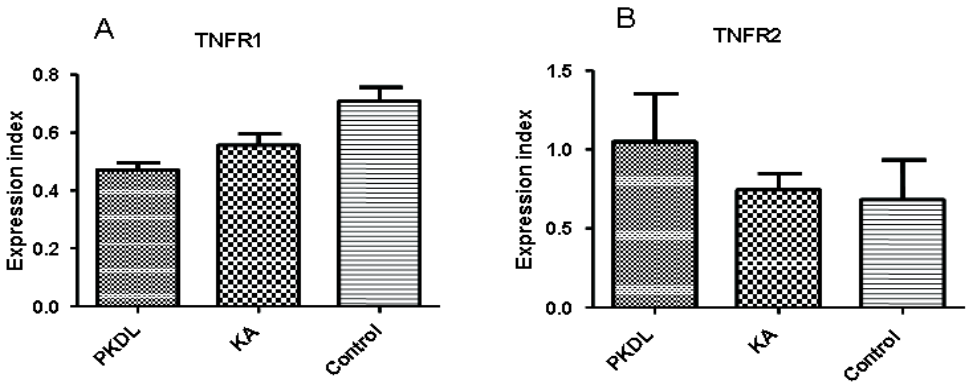


Fig. 17: Transcripts of Tumor necrosis factor receptor-1 [TNFR1] (A) and Tumor necrosis factor receptor-2 [TNFR2] (B) in lesion tissues from patients with PKDL (n=14) and KA (n=10) and control tissues (n=6).

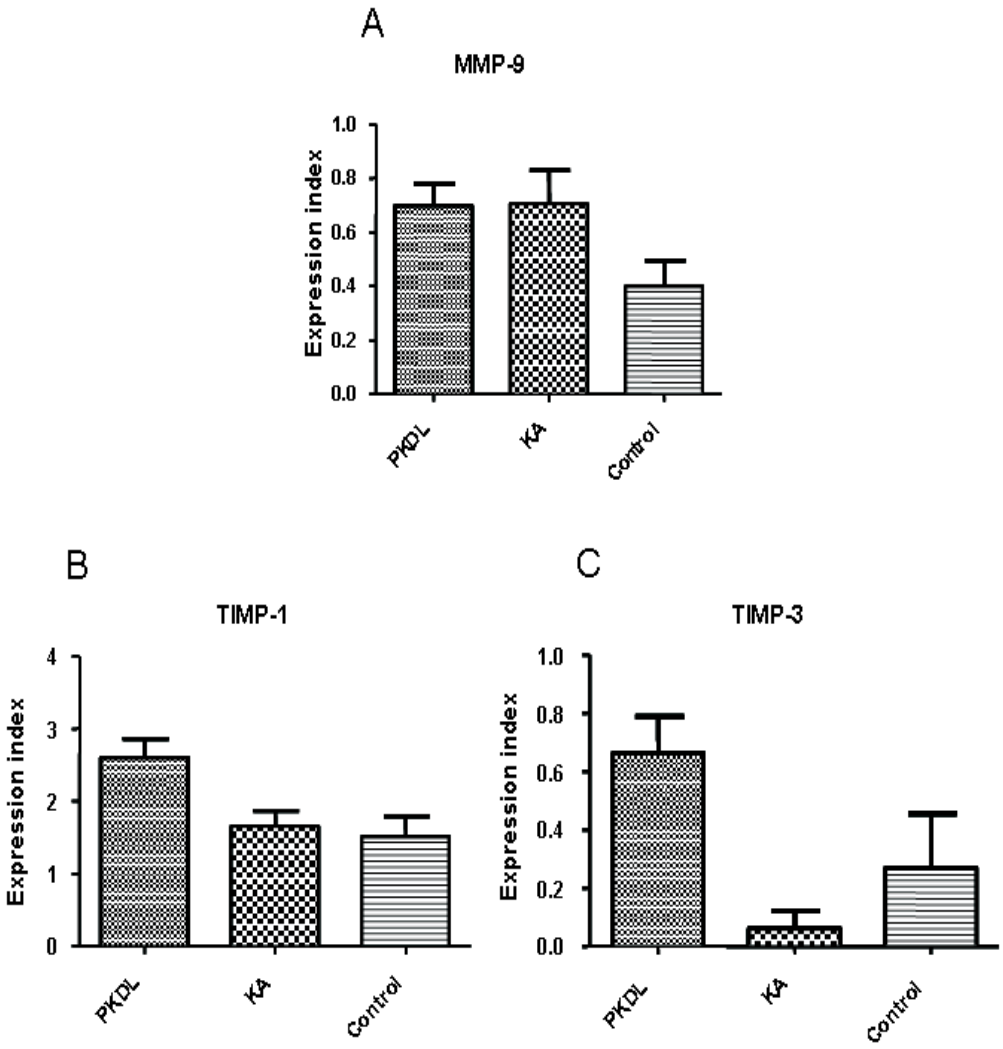


Fig. 18.: Transcripts of Matrix metalloproteinase-9 (A), Tissue inhibitor of matrix metalloproteinase [TIMP] -1 (B) and TIMP-3 (C) in lesion tissues of patients with PKDL (n=14) and KA (n=10) and control tissues (n=6).

Analysis of host immunodetermnants in PKDL lesions

In the previous report, we had reported genes showing modulated expression in PKDL compared to KA using cDNA array technology. Here, we compare gene expression in PKDL lesion tissues with healthy tissue using cDNA array. Real time PCR was used to validate the array results.

Microarray analysis: Hybridization, autoradiograph and analysis of data

Details of experiments have already been given in previous year report. In brief, RNA from control tissue was used to synthesize radio-labeled probe. cDNA was prepared in the presence of 50 µCi of α-^{33}P dATP and 268 gene specific primers for each gene represented on the array. Using Scintillation Counter, $5x10^6$ cpm of each cDNA probe was used for hybridization. Atlas array membranes were prehybridized at 68°C for 1 h with ExpressHyb pre-hybridization solution containing 100 µg of sheared salmon testis DNA/ml. Probes were added to separate nylon arrays and allowed to hybridize overnight at 68°C under identical hybridization conditions. After washing, membranes were exposed to phosphor screen and images were captured with Phosphoimager Typhoon. Imagequant software was used for analysis of images. The ratio of gene expression levels was determined by dividing the mean signal intensity (average of 3 array experiments) of PKDL with control. Differential gene expression was considered significant when the average ratio was greater than 2.0 when comparison was performed between disease and control. A comparative assessment of gene expression in lesions tissue revealed that 50 (18.6%) genes in PKDL showed 2.0 fold higher expressions compared to control, as shown in Table 2. Genes were categorized in three groups based on gene characteristics, viz. group 1 (cytokines and chemokines), group 2 (receptors) and group 3 (others, comprising of signaling molecule, transcription factor, apoptotic genes, etc).

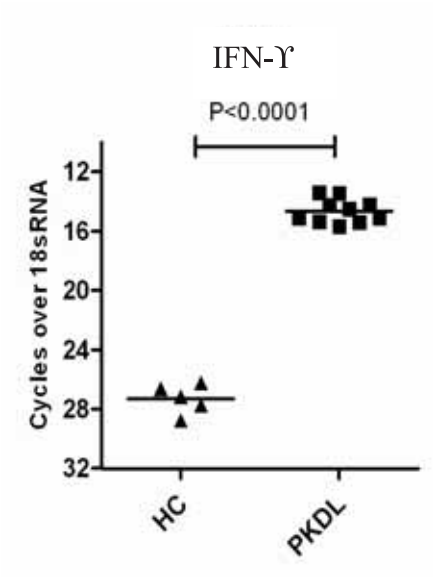
Validation of micro-array data

2μg RNA was reverse transcribed using High capacity cDNA preparation kit. cDNA synthesis was performed in a DNA thermal cycler and 100 ng of cDNA was used for real time PCR amplification. PCR was performed in an ABI PRISM 7900HT Sequence Detection System using TaqMan Universal PCR Master Mix and cDNA specific FAM-MGB labeled primers sets for IFN-γ, TNF-α, IL-10, TGF-β, MCP-1, IL-17 and CD40 with18S rRNA as control for the relative amount of mRNA in each sample. The relative quantification of products was determined by the number of cycles over 18S rRNA endogenous control required to detect the gene expression of interest (Fig. 19). Further analysis revealed that the expression of all genes increased significantly ($P < 0.0001$) in the PKDL group compared to the control.

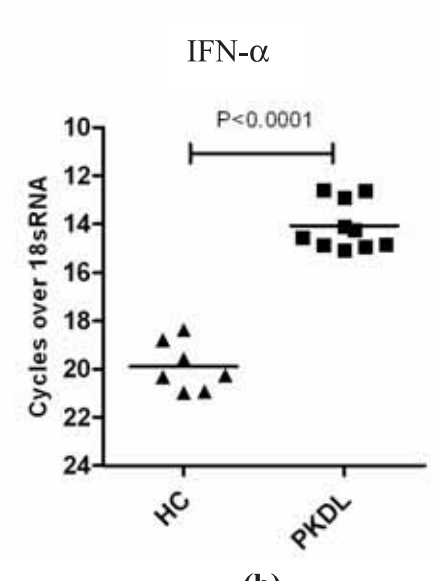
Table 2: Genes showing higher expression in dermal lesion tissue of PKDL patients compared to normal skin (HC) of healthy individuals

Gene Accession No	Gene description	Fold Change PKDL/HC
Cytokines & Chemokines X01394	Tumor necrosis factor alpha (TNF-α)	9.0
M24545	Small inducible cytokine A2 (MCP-1)	7.33
L06801	Interleukin 13	5.64
J04130	Small inducible cytokine A4	5.15
K02770	Interleukin 1, beta	5.11
U32659	Interleukin 17	5.0
M23452	Small inducible cytokine A3 (MIP-1α)	4.83
X17543	Interleukin 9	3.88
M57627	Interleukin 10	3.84
L07414	Tumor necrosis factor (ligand) superfamily, member 5	3.33
M65290	Interleukin 12B	3.0
J03241	Transforming growth factor, beta	2.95
X04688	Interleukin 5	2.94
X53799	GRO2 oncogene	2.91
J04156	Interleukin 7	2.88
M77349	Transforming growth factor, beta-induced, 68kD	2.79
X04602	Interleukin 6 (interferon, beta 2)	2.68
U14407	Interleukin 15	2.65
M13982	Interleukin 4	2.61
Y00787	Interleukin 8	2.58
D12614	lymphotoxin alpha	2.5
X01992	Interferon, gamma	2.36
X01992	IK cytokine, down-regulator of HLA II	2.24
U57059	Tumor necrosis factor (ligand) superfamily, member 10	2.24
A14844	Interleukin 2	2.22
X02851	Interleukin 1, alpha	2.13

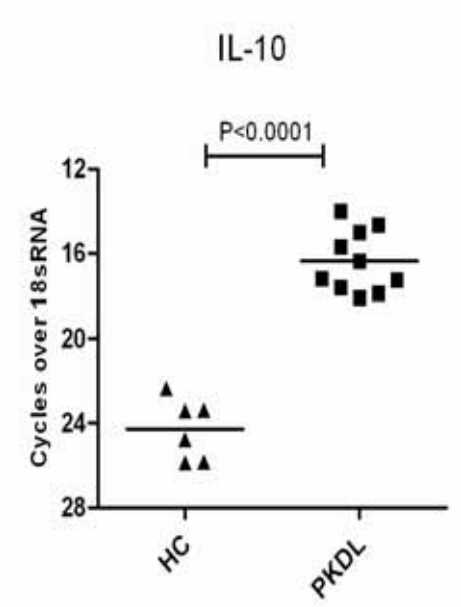
Receptors D11086	Interleukin 2 receptor, gamma	6.0
X77722	Interferon (alpha, beta and omega) receptor 2	4.4
M84747	Interleukin 9 receptor	3.96
AF016268	Tumor necrosis factor receptor superfamily, member 10b	3.94
M29696	Interleukin 7 receptor	3.76
X60592	Tumor necrosis factor receptor superfamily, member 5 (CD40)	3.66
D10925	Chemokine (C-C motif) receptor 1	3.4
X52425	Interleukin 4 receptor	2.80
X12830	Interleukin 6 receptor	2.70
U90875	Tumor necrosis factor receptor superfamily, member 10a	2.70
M63099	Interleukin 1 receptor antagonist	2.52
M26062	Interleukin 2 receptor, beta	2.46
Z70519	Tumor necrosis factor receptor superfamily, member 6	2.20
Others D17517	TYRO3 protein tyrosine kinase	13.07
X14454	Interferon regulatory factor 1	8.07
M21626	T cell receptor alpha variable 4	5.44
D38122	Tumor necrosis factor (ligand) superfamily, member 6 (FASL)	4.08
X02492	Interferon, alpha-inducible protein	3.5
L08187	Epstein-Barr virus induced gene 3	3.42
M99437	Notch homolog 2 (Drosophila)	2.76
M76125	AXL receptor tyrosine kinase	2.62
U33635	PTK7 protein tyrosine kinase 7	2.59
AF022385	Programmed cell death 1	2.08


IFN-ϒ
P<0.0001
Cycles over 18sRNA
12
16
20
24
28
32
HC
PKDL

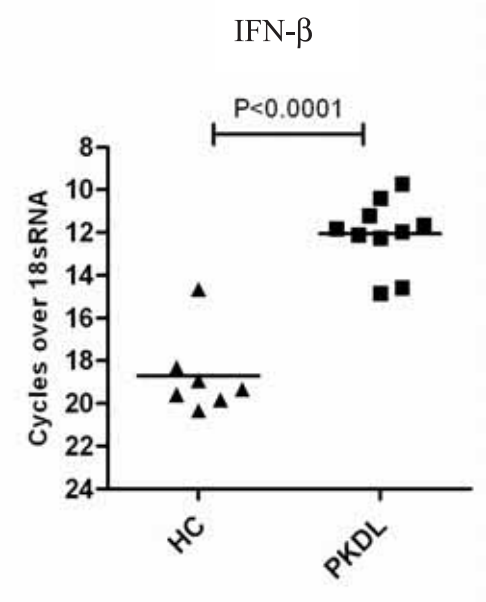
(a)


IFN-α
P<0.0001
Cycles over 18sRNA
10
12
14
16
18
20
22
24
HC
PKDL

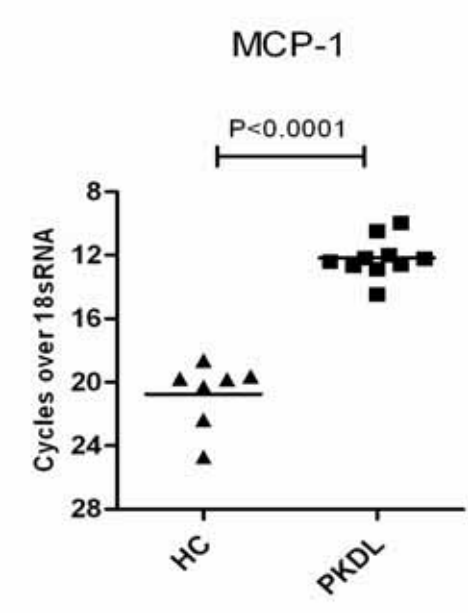
(b)


IL-10
P<0.0001
Cycles over 18sRNA
12
16
20
24
28
HC
PKDL

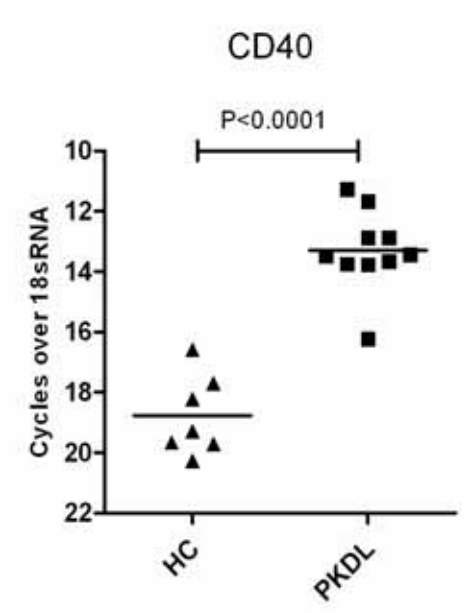
(c)


IFN-β
P<0.0001
Cycles over 18sRNA
8
10
12
14
16
18
20
22
24
HC
PKDL

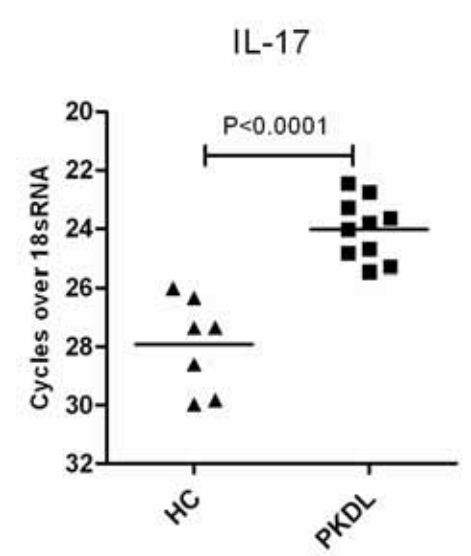
(d)



(e)



(f)



(g)

Fig. 19: Relative mRNA levels of IFN-γ (a), TNF-α (b), IL-10 (c), TGF-β (d), MCP-1 (e), CD40 (f) and IL-17 (g) in lesion tissue of PKDL and normal skin of healthy individuals. Gene expressions were quantified using real time RT-PCR. Values were normalized to 18S rRNA expression.

Future work

The microarray analysis will be extended for assessment of gene expression in KA in comparison with control levels. Further, the microarray data will be validated at protein level by western blotting/ immuno-histochemistry. Pathway based real time PCR assay will be used to understand the defects in signaling pathways.

4. ANALYSIS OF LOCALIZED AND CIRCULATING IMMUNE RESPONSES IN PATIENTS OF CUTANEOUS LEISHMANIASIS CAUSED BY *LEISHMANIA TROPICA*

Scientific Staff	:	Dr. Poonam Salotra, Dr. Rajesh Kumar, Anand Raj
Collaborators	:	Dr. R. A. Bum, Safdarjang hospital
Duration	:	2008 - 2011

Aims, Objectives and Background

In India, CL is endemic in Western Thar region of Rajasthan particularly in Bikaner region, where we have recently established *Leishmania tropica* as the causative agent.

Extensive studies with experimental models have shown that the outcome of *Leishmania* infection is critically dependent on the activation of one of the two subsets of CD4 T-cells, Th1 and Th2. Interferon (IFN)-γ, secreted by Th1 cells, leads to host resistance to infection with *Leishmania* parasites, whereas interleukin-4 (IL-4), secreted by Th2 cells, is associated with down-modulation of IFN-γ-mediated macrophage activation. However, in human CL, a clear functional dichotomy in CD4 T-cells has not definitely been documented. In this context, a few studies have analyzed the intralesional cytokine gene expression in various forms of CL. In CL due to *L. braziliensis*, IFN-γ was preferentially expressed in localized lesions, whereas IL-4, IL-5, and IL-10 were detected in mucosal and diffuse forms of the disease; however, in patients infected with *L. mexicana*, high level of IL-10 and IFN-γ were expressed. In humans, polymorhonuclear (PMN) cells containing *Leishmania* start secreting chemokines such as IL-8 (also known as CXCL8) that are essential in attracting PMNs to the site of infection. Upon

experimental infection with *L. major*, MIP-2 and keratinocyte-derived cytokine (KC; also known as CXCL1), the functional murine homologs of human IL-8, are rapidly produced in the skin. Lesions in CL patients contain high levels of CC chemokine ligand (CCL2/MCP-1), CXCL9/Mig, CXCL10/IFN-γ-inducible protein (IP-10), whereas patients with DCL express CCL3/MIP-1α. Thus, the levels of cytokines/chemokines are modulated differently depending on the clinical forms of the disease and the causative species of *Leishmania*.

While *L. major* has become a favourite model for immunologists, the mammalian response to *L. tropica* has never been studied thoroughly and an open field awaits the intrepid investigator. Our aim was to understand the localized and circulating immunological response before and after therapy in patients of CL. This study led to identification of key cytokines that determine the clinical outcome of the disease and helps in understanding the immunological pathways that may be involved in the pathogenesis of CL caused by *L. tropica*.

Work done during the period

The association between localized and circulating levels of immune-determinants in patients of CL was evaluated in the present study. Cytokines in dermal lesions of CL patients at pre-treatment (n=31) and post-treatment stage (n=14) were analyzed by RT-PCR. Circulating levels of cytokines were determined by Cytokine Bead Array and ELISA.

Intralesional expression of cytokines in CL patients

Intralesional expression of interferon (IFN)-γ, tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-8, IL-10 and IL-4 mRNA was analyzed by RT-PCR in CL patients (n=31), and healthy controls (n=6). Transcripts of IFN-γ, TNF-α, IL-1β, IL-8, and IL-10 were expressed in lesions of all the CL patients, while IL-4 was detected in 77.4 % (24/31) biopsies. The expression levels of all cytokines were significantly elevated in CL lesions, compared with those in control tissues ($P<0.001$ for all cytokines) (Fig. 20A). IL-1β was expressed at a very high level compared to other cytokines in all the samples. Comparative analysis in mRNA transcript level of all the cytokines was carried out in early lesions (≤2 months, n=14) and late lesions (≥ 3 months, n=15). Level of IL-4 was significantly higher in early lesions ($P<0.05$) compared to late lesions (Fig. 20B), while other cytokines were at comparable levels.

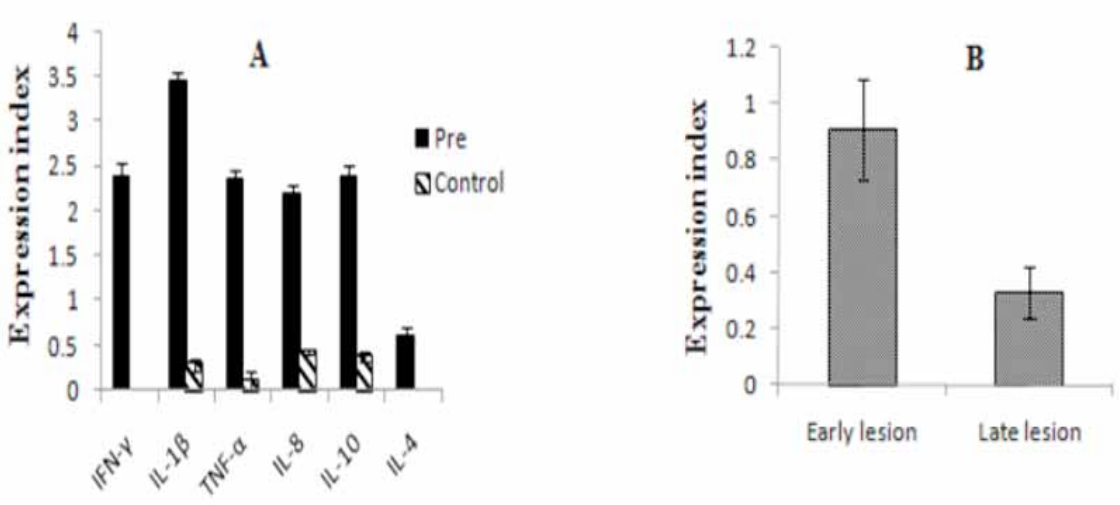


Fig. 20: Analysis of intralesional cytokines in CL patients at pre-treatment stage. A. mRNA Levels of interleukins [IL-1β], tumor necrosis factor [TNF]-α and interferon [IFN]-γ in dermal lesion of CL patients at pre-treatment stage (n=31) and healthy controls (n=6). B. Comparative assessment of IL-4 mRNA expression in early lesions (<2 months, n=14) and in late lesions (≥2 months, n=15) of CL patients. Normalized cDNA was amplified with respective cytokine primers. Polymerase chain reaction products were electrophoresed, and the intensity of signal was determined by densitometry. The graph shows results as an expression index, defined as the ratio of the intensity of cytokine with respect to the HPRT gene. The bars indicate SE. P <0.05 was considered to be statistically significant.

Analysis of intralesional cytokines in CL patients before and after treatment

Paired samples were collected from nine patients at post-treatment stage for comparative analysis of cytokine mRNA levels. Of these, three were treated with SAG, five were treated with RFM and one was given SAG followed by RFM. A significant decrement in the expression levels of IFN-γ, TNF-α, IL-1β, IL-8, IL-10 and IL-4 mRNA was noticed after treatment (P<0.05 for levels of all cytokines) (Fig. 21A). mRNA expression of MCP-1 and iNOS was significantly up-regulated in CL patients at pre-treatment stage compared to healthy controls (P<0.001 and P<0.05 respectively), but remained high at post-treatment stage (p>0.05) (Fig. 21A). mRNA levels for IFN-γ, TNF-α, IL-1β, IL-8, IL-10 and IL-4 were comparatively analyzed in lesions of CL patients treated with SAG or RFM (Fig. 21B). Three patients treated with SAG and five treated with RFM could be followed in this study. Additional three patients treated with SAG and two treated with RFM, where tissue lesions at pre-treatment stage were not available, were also included to obtain more significant data. There was significant decrement in the

levels of cytokine gene expression in the CL lesions treated with RFM ($P<0.05$), whereas no significant decrement was noticed in the levels IFN-γ, TNF-α and IL-10 ($P>0.05$) in lesions treated with SAG.

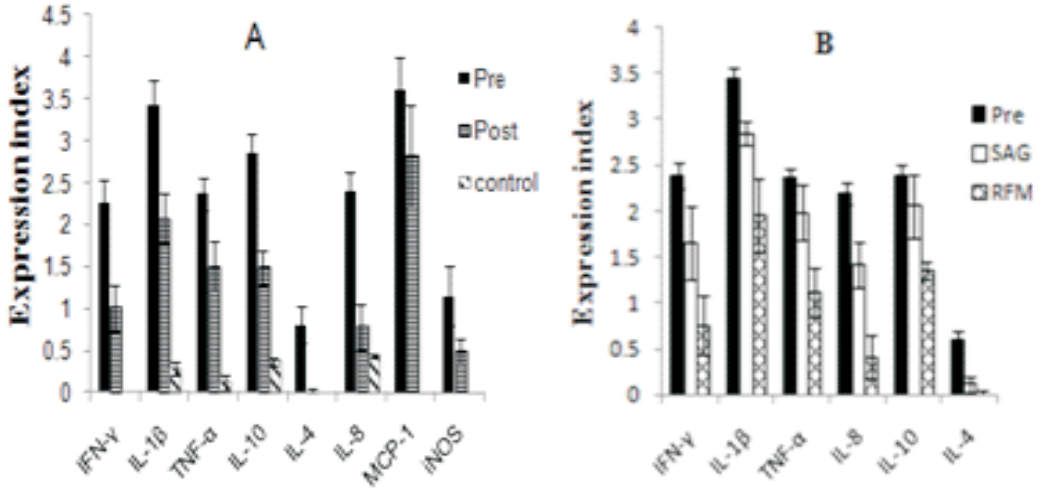


Fig. 21: Comparative analysis of intralesional immunodeterminants in CL patients at pre-treatment stage and at post-treatment stage .A. mRNA Levels of cytokine IL-1β], TNF-α, IFN-γ, monocyte chemotactic factor [MCP] and inducible nitric oxide [iNOS] in paired lesion tissues from CL patients at pre-treatment (n=9), post-treatment stage (n=9) and in healthy controls (n=6). B. Levels of cytokine transcripts in lesion tissues from CL patients at pre-treatment stage (n=31) and after treatment with sodium stibogluconate (SAG, n=6) or rifampicin (RFM, n=7). Normalized cDNA was amplified with respective cytokine primers.

Cytokine levels in sera of CL patients by Cytokine Bead Array

In order to understand the *in vivo* circulating cytokine profile, serum cytokine levels were analyzed at pre-treatment stage and post-treatment stage in CL patients and compared with healthy controls. Level of IL-8 was found to be significantly higher in CL samples at pre-treatment stage (1022.4 ± 313.78 pg/ml) compared to post-treatment stage (10.11± 6.97 pg/ml) or control (10.48±3.9 pg/ml). The level of IL-8 was restored to normal level after treatment (Fig. 22). The levels of other circulating inflammatory cytokines examined, including IL-1β, IL-6, IL-10, TNF-α, and IL-12p70 were not detectable in sera.

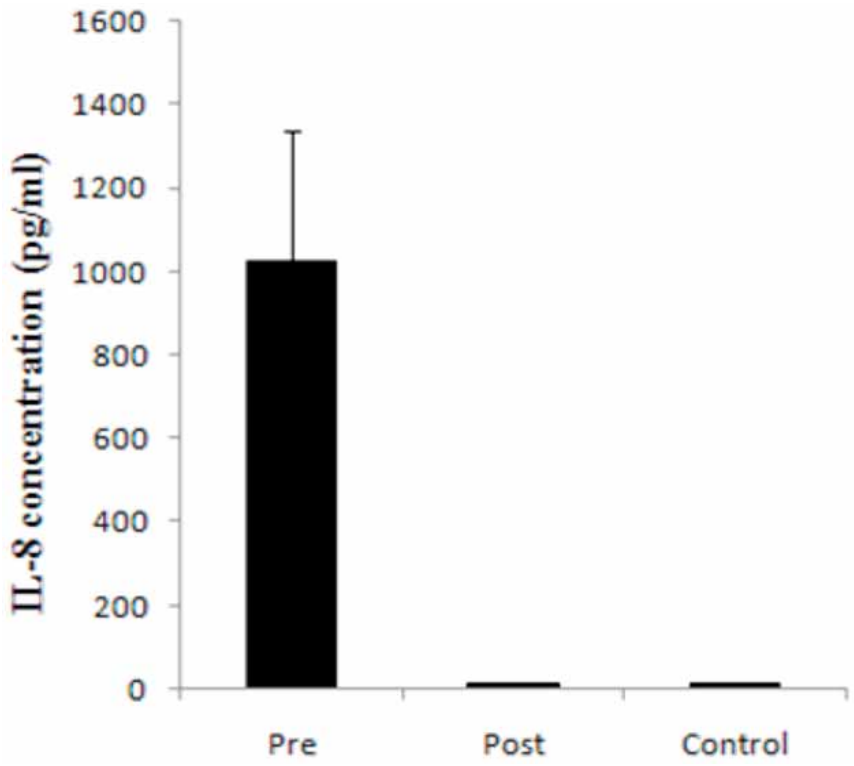


Fig. 22: Quantitative estimation of interleukin [IL]-8 in sera of CL patients by cytokine bead array analysis (CBA). Analysis of IL-8 at protein level was carried out at pretreatment (n=15), and post-treatment stage (n=9) and in healthy controls (n=4). Values are mean ± SE. The bars indicate SEs.

Measurement of IL-8 and MCP-1 by ELISA in sera of CL patients

To establish the association between the circulating and localized response of IL-8 and MCP-1, quantitative analysis of IL-8 and MCP-1 was carried out at pre-treatment stage (n=20) and post-treatment stage (n=9) in sera samples of CL patients and compared with healthy control sera (n=9) (Fig. 23). Since IL-8 was detected at a significant level by CBA, its concentration was further evaluated by the more sensitive ELISA method. Level of IL-8 determined in sera (1:20 dilution) was found to be significantly higher ($P<0.001$) in CL patients (20/20) at pretreatment stage (89.04±18.8 pg/ml) compared to post-treatment stage (13.12±5.16 pg/ml) or control (5.16±1.45 pg/ml). Similarly, elevated level of MCP-1 was observed in all 20 CL cases at pretreatment stage (39.25±5.29pg/ml) compared to control (21.1±2.6pg/ml, $P<0.01$) which remained high at post-treatment stage (47.77±3.03 pg/ml, $P>0.05$).

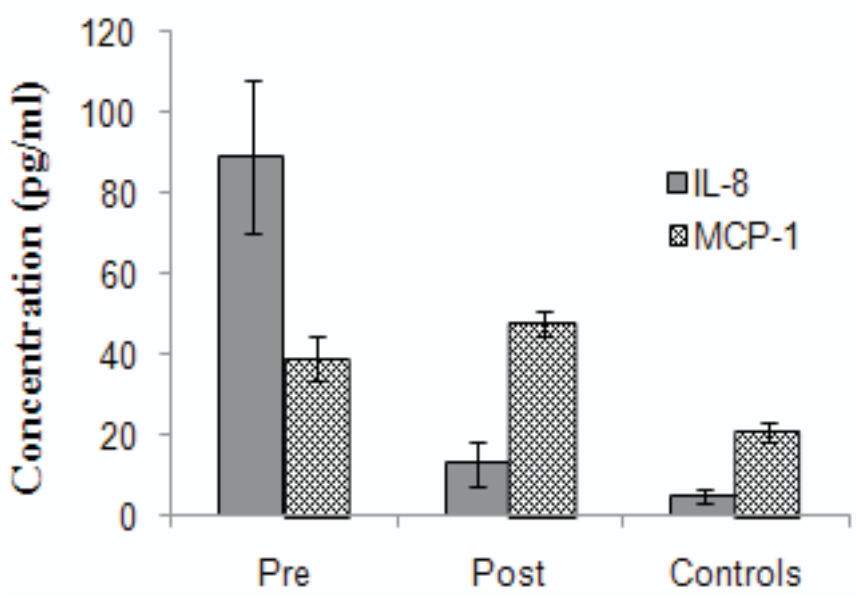


Fig. 23: Quantitative analysis of IL-8, monocyte chemoattractant protein (MCP)-1 and in sera of CL patients by ELISA. A. Levels (pg/ml) of Interleukin [IL]-8 and monocyte chemotactic factor [MCP]-1 in 1:20 dilution of sera at pretreatment (n=20), and post-treatment stage (n=9) in patients and controls (n=9).

Quantitative assessment of NO in sera samples

Circulating NO level was analyzed at pre-treatment stage in CL patients (n=32) and healthy controls (n=10), followed by evaluation at post-treatment stage (n=10) (Fig. 24). The level of nitrite was found to be significantly higher in CL samples at pre-treatment stage (61.37±2.46 µM) as compared to healthy controls (15.4±0.99 µM, $P<0.001$) while it was not significantly down-regulated after treatment (41.1±10.11 µM, $P>0.05$).

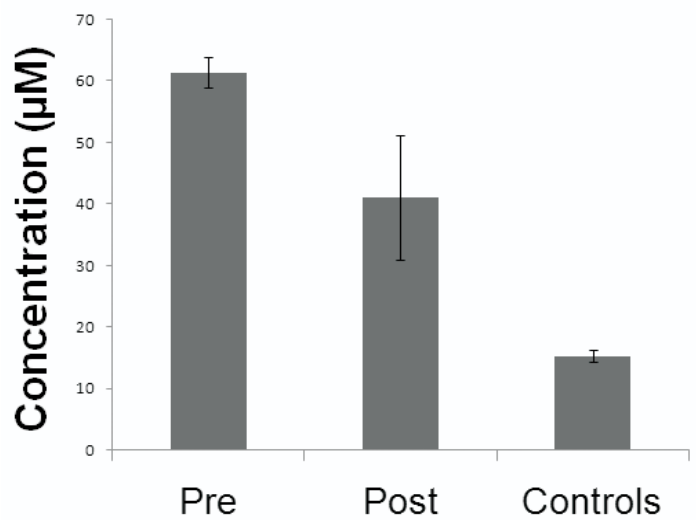


Fig. 24: Measurement of serum nitrite level (µM) at pretreatment (n=32), and post-treatment stage (n=10) in Indian CL patients and controls (n=10) by calorimetric assay. Values are mean± SE. The bars indicate SEs.

Future work

The production of IL-8 in CL patients augments the enhanced influx of PMN serving as parasite "shelter" by delaying the apoptosis of PMN, therefore, chemotaxis and apoptotic pathway analysis will be determined. Further study will be carried out to capture the global picture of cytokine gene expression in CL patients using microarray which includes various cytokine/chemokines and its receptor genes, apoptotic genes, etc. The defect in signaling mechanism upon infection will be evaluated exploiting signaling pathway based on Real-Time PCR assay followed by investigation of key molecules at protein level.

ADULT STEM CELL BIOLOGY

ADULT STEM CELL BIOLOGY

1. OPTIMAL ATTENUATION CONDITIONS FOR 3T3 FIBROBLASTS FOR USE AS FEEDER CELLS

Scientific Staff	:	Dr. L.K. Yerneni, Ashok Kumar
Technical Staff	:	Bijender
Duration	:	2006-2009

Aims, Objectives and Background

The primary adult keratinocytes and embryonic epithelial stem cells require mesenchymal interactions for their proliferation under normal culture conditions. Such cultures are established using mesenchymal cells such as mouse fibroblasts that serve as feeder cells to epithelial cultures. Application of attenuated feeder cells in co-culture system is considered as an integral part of cell cloning and replication. There is a choice of attenuating feeders with either Gamma-irradiation or Mitomycin C, which is often guided by the facts that Gamma-irradiation equipment and its establishment is complex and expensive while Mitomycin C is very economical. However, there has been a controversy regarding accuracy use of these two methods. It appears that the controversy around the Mitomycin C induction of attenuation remains associated with the reported wide range of Mitomycin C doses from 4 – 10 µg/ml. It is not clear from the existing data on this subject whether the range of Mitomycin C doses would yield similar extent of attenuation with equivalent metabolic states. Hence it is planned to estimate the correct exposure conditions for Mitomycin C induction of feeder cell attenuation and to verify the influence of differentially growth arrested feeders on epidermal keratinocyte growth in a co-culture.

Work done during the period

In order to estimate the ability of differentially growth arrested 3T3 cells by numerical doses of A-p, A-q, A-r, B-p, B-q, and B-r in supporting the growth of target stem cells, experiments were conducted by seeding into 24 well plates the primary human epidermal keratinocytes (*Genlantis*, USA) and differentially growth arrested 3T3 cells. The untreated 3T3 cells and those treated with a potent lethal dose (C-p) served as negative and positive controls, respectively,

for comparison. Periodical viable cell counts were performed on separately collected 3T3 and keratinocytes.

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.

Both foolproof irreversible growth arrest and optimal stimulation of keratinocyte proliferation were experimentally demonstrated to depend upon numerical dosing of Mitomycin C. The observations were drafted as patent document under New Invention Report entitled "A culture system for the growth of stem cells". The draft is being fine-tuned for filing at the patent office by the authorized attorney through the Intellectual Property Rights Division, ICMR.

In the meantime, based on results obtained from this study so far an ad-hoc research project entitled "*A novel arithmetic approach for fool-proof production of growth arrest in 3T3 cells suitable for human epidermal culture*" has been submitted to ICMR under the priority area of "*in vitro* model using biotechnology in Pharmacology". The project is now being evaluated for funding under Cell and Molecular biology. The project is aimed at improved feeder functionality with reference to *in vitro* growth characteristics of human keratinocytes using mytomicin C as an alternative to the expensive γ-irradiation- induced attenuation.

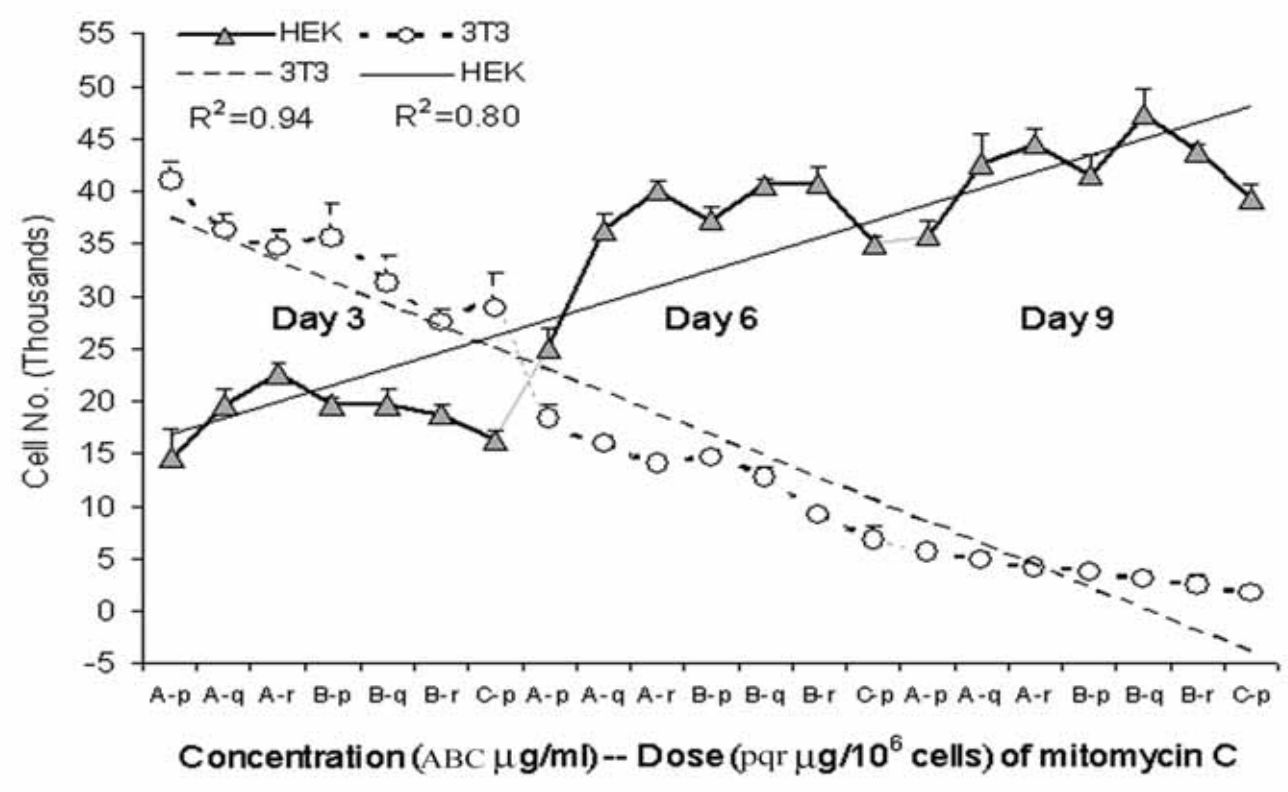


Fig. 1: Line diagram plotted with number of keratinocyte stem cells and 3T3 cell number obtained with 3T3 cells attenuated with concentration-dose combinations of A and B g Mitomycin C at doses of p, q and r g and C g Mitomycin C at a dose of p g for a 2-hours pulse as represented by A-p, A-q, A-r, B-p, 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 y-axis.

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

Scientific Staff	:	Dr. L.K. Yerneni, Rishi Man Chug
Collaborators	:	Dr. Abraham Samuel, (NCRM), Chennai Prof. Yuichi Mori, Dr. Hiroshi Yoshioka, Mebiol Gel Incorporation, Japan
Duration	:	2007-2010

Aims, Objectives and Background

In recent times, several advances have been made towards wound healing involving *in vitro* construction of 3-dimensional cellular structures using various matrix materials. Such tissue engineering constructs utilize certain biological matrices like collagen, proteoglycans, hyaluronic acid, laminin, fibronectin, etc. However, several complex safety issues still remain associated with such materials that are either animal sourced or semi-synthetic. A purely synthetic non-toxic thermoreversible gelation polymer hydrogel, Mebiol gel developed by Prof.Yuichi Mori at the Waseda University, Tokyo, Japan, transforms into liquid at low temperature and solid upon heating. This patented gel has been proven to support organotypic organization of hepatic progenietor cells, corneal limbal cells, mesenchymal stem cell to osteogenesis and pancreatic islets, in addition to supporting the growth of several cells. However, Mebiol gel has not been tested to sustain the growth of epidermal and dermal cells. Hence, it is proposed to determine the ability of Mebiol gel, a synthetic thermo-reversible hydrogel polymer to support without feeders the growth and differentiation of normal human skin keratinocyte stem cells.

Work done during the period

The established primary keratinocytes were obtained from *Genlantis, USA*. Initial experiments involved adult human epidermal keratinocytes. The attenuated stocks of NIH 3T3 cells were raised by growth arrest as per our established attenuation protocol using Mitomycin C. The

experiments were performed using TGP dissolved in Keratinocytes Medium (KCM) and human adult keratinocytes were seeded into the constituted gels into the wells of 48 well-plate in the following variable manner.

1. Seeding Keratinocytes over the plastic followed by an overlay of TGP in KCM.

2. Seeding Keratinocytes and attenuated 3T3 fibroblasts over the plastic followed by an overlay of TGP in KCM after they properly adhered.

3. Seeding keratinocytes over TGP in medium.

4. Keratinocytes and attenuated 3T3 seeded over TGP in KCM.

5. Keratinocytes seeded over TGP in KCM that was mixed with attenuated 3T3.

Appropriate controls for comparing the efficiency of growth support of TGP with that of conventional culture surface were maintained. Non-attenuated 3T3 were plated as parallel controls for comparison. All the cells were seeded at a uniform density of 10^4 cells per cm^2. The cultures were incubated in KCM with 10% fetal calf serum under normal cell culture conditions as per our previous standard protocol. The incubation of triplicate wells continued for 20 days with observations made every alternate day. At the end of incubation period, the gels were cooled to sol state and the cells were collected by centrifugation and counted after vital staining by Trypan Blue.

It was found that 3T3 cells either over TGP or 3-dimensionally suspended in TGP remained rounded (Fig. 1) throughout the incubation period, or clumped together over a period of time (Fig. 2), while they remained well spread out if the gel was poured over the firmly attached cells on plastic surface. However, the live attenuated 3T3 cell count at the end of experiments was reduced to 40% of initial count while that of non-attenuated 3T3 remained more or less constant. On the other hand, the non-attenuated 3T3 over plastic (control) became confluent after 6 days of incubation and the live attenuated 3T3 (control) cells were reduced to 38%.

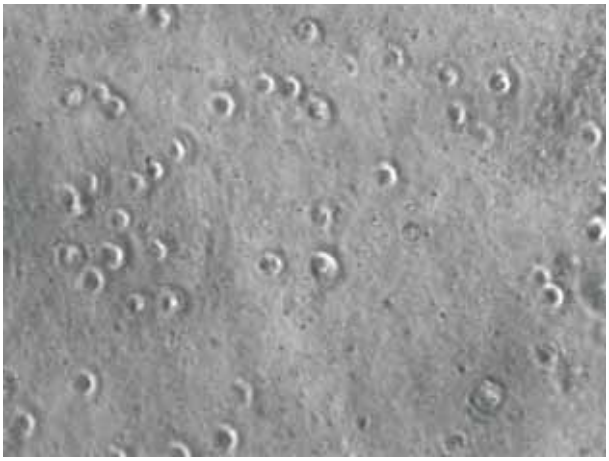


Fig. 1: Growth arrested NIH 3T3 fibroblasts embedded in 3-D Mebiol gel showing rounded but healthy morphology. Phase contrast 100 X.

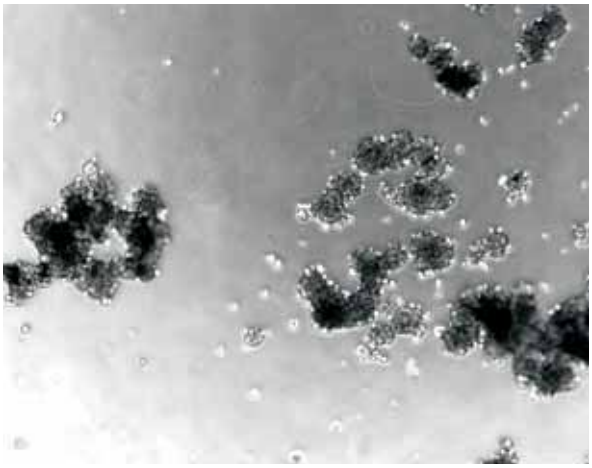


Fig. 2: Growth arrested NIH 3T3 cells plated along with human human epidermal keratinocytes over the surface of Mebiol gel showing clumping of 3T3. The attached single cells in the background are the keratinocytes. Phase contrast 50X

The experiments suggested that TGP arrests the growth of 3T3, which is in a way a favorable sign for its potential use in establishing purer cultures of keratinocytes from the skin biopsy by weeding out the contaminating dermal fibroblasts.

On the other hand, the number of epidermal keratinocytes in wells with TGP gelated over them remained unaltered. When the keratinocytes were plated over TGP, most of the cells were round and loosely adhered to the gel (Fig. 3). Towards the end of incubation period of 20 days, the cell number was reduced and a few keratinocytes with apparent cytoplasmic expanses (Fig. 4) were found migrating over the surface of TGP. These observations are in contrast to the formation of well defined colonies by the keratinocytes plated with attenuated 3T3 fibroblasts over the plastic surface (Fig. 10 B).

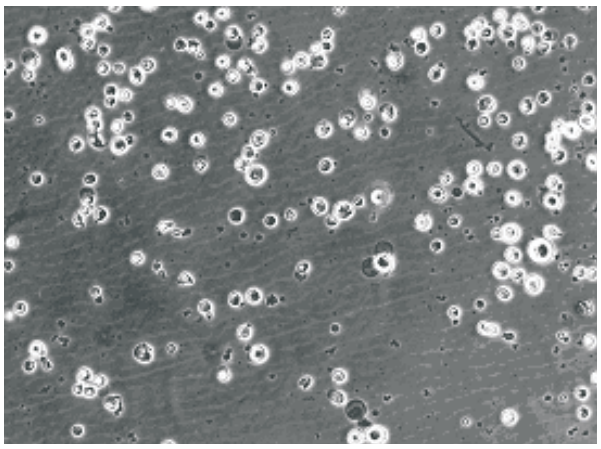


Fig. 3. Large number of human adult epidermal keratinocyte stem cells plated over the gelated TGP on day 2. Phase contrast 50 X.

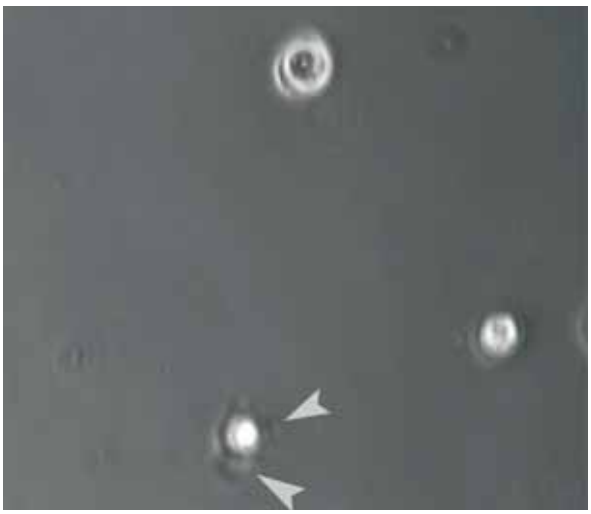


Fig. 4: Few human adult epidermal keratinocytes stem cells remained attached over the gelated TGP 20 days after they were plated. The cells with cytoplasmic expanses into the gel (Arrows) were found migrating. Phase contrast 250X.

Human Epidermal Keratinocyte growth was tested with Mebiol gel over the keratinocytes that were allowed to adhere to plastic surface of culture dish. In brief, keratinocytes were plated in 48-well plate and allowed to anchor for 24 hours before being overlaid by Mebiol gel. Specifically demarcated regions were photographically monitored every day over a period of 10 days at the end of which cells were collected by trypsinization and viable cells counted. The movements of small-sized keratinocytes were observed (Fig. 5-6).

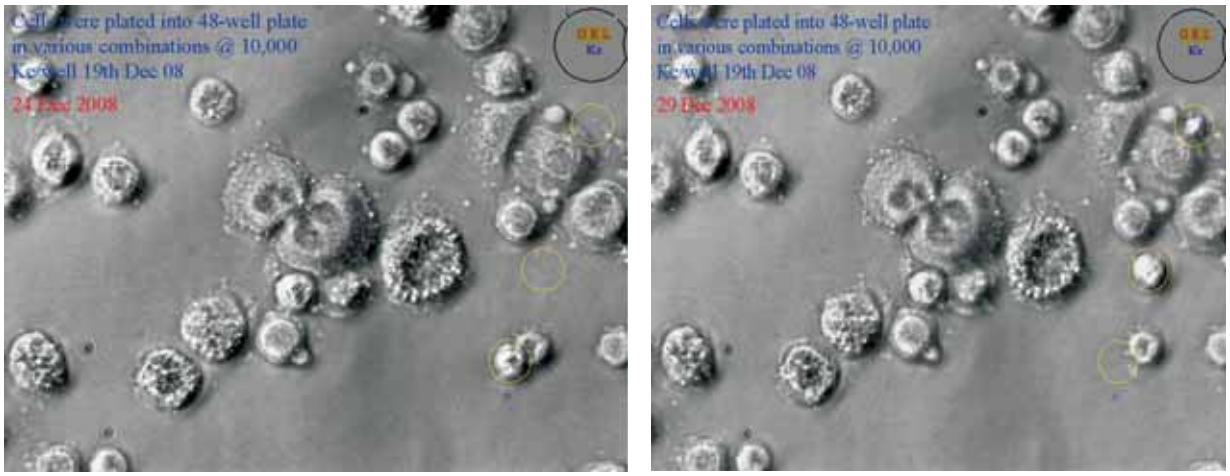


Fig. 5: Keratinocytes plated over plastic surface of 48-well plate at 10, 000 cell density per well, showed migration (encircled locations) of smaller cells. Left image: Day 1; Right Image: Day 5 after being overlaid with the gel.

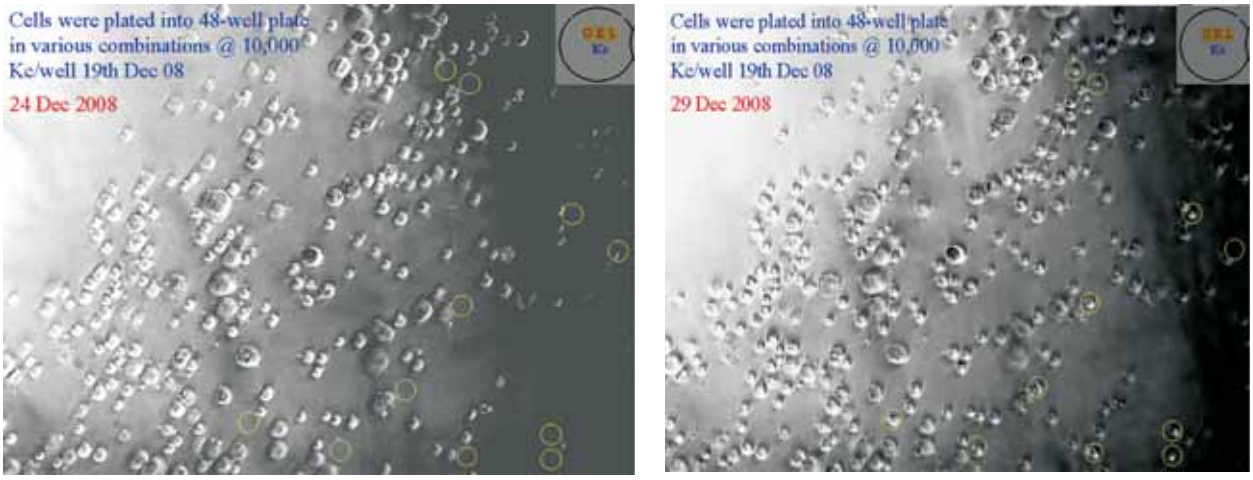


Fig. 6: Keratinocytes plated over plastic surface of 48-well plate at 10, 000 cell density per well showed several new appearances (encircled) of smaller cells suggesting probable cell proliferative activity in smaller keratinocytes. Left image: Day 1; Right Image: Day 5 after being overlaid by the gel.

However, there was 50 per cent decline in viable cell yield. A differential count indicated that almost all the viable cells were of small size and larger cells constituted the dead. The results were suggestive of proliferation and migration of smaller sized keratinocytes while larger keratinocyte population showed poor survival in Mebiol gel.

It is felt that the keratinocytes could possibly be stimulated to proliferate only when they are enveloped by the TGP, since the surface anchorage was found to be poor resulting in the loss of cells. Hence, it is being planned to place the keratinocytes sandwiched between two layers of TGP gelated successively.

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 the cells seeded as concentrated pellet in between 2 discs of gels in cell inserts showed marked migrations towards the periphery of the discs (Fig. 7).

The cell counts indicated significant increase in predominantly small cell population compared to either only keratinocytes or keratinocytes with attenuated 3T3 (Fig. 8). On the other hand, keratinocytes seeded over conventional culture surface without 3T3 serving as control showed cytoplasmic enlargement indicating terminal differentiation (Fig. 9 A) and those plated over attenuated 3T3 fibroblasts formed colonies (Fig. 9 B). The results are indicative of selective proliferation of keratinocytes of smaller size in Mebiol gel.

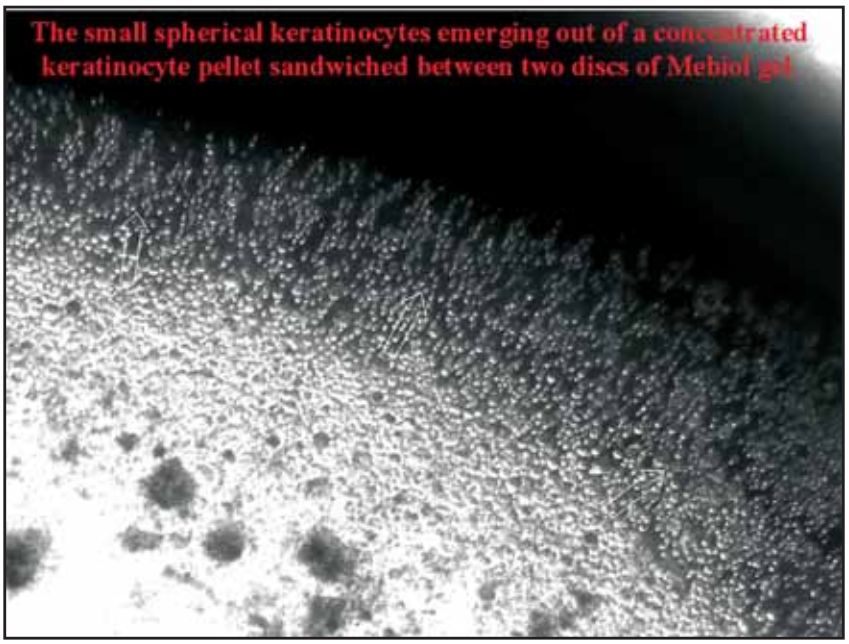


Fig. 7: The small spherical keratinocytes (arrows) emerging out of a concentrated keratinocyte pellet sandwiched between two discs of Mebiol gel.

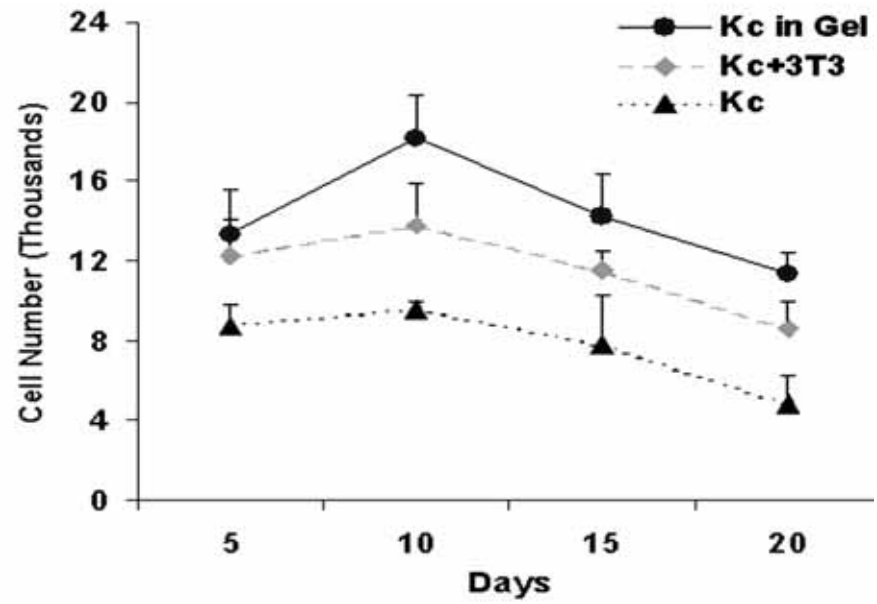


Fig. 8: Human epidermal Keratinocyte growth in Mebiol gel compared to Rheinwald-Green feeder dependant culture and non-feeder culture systems.

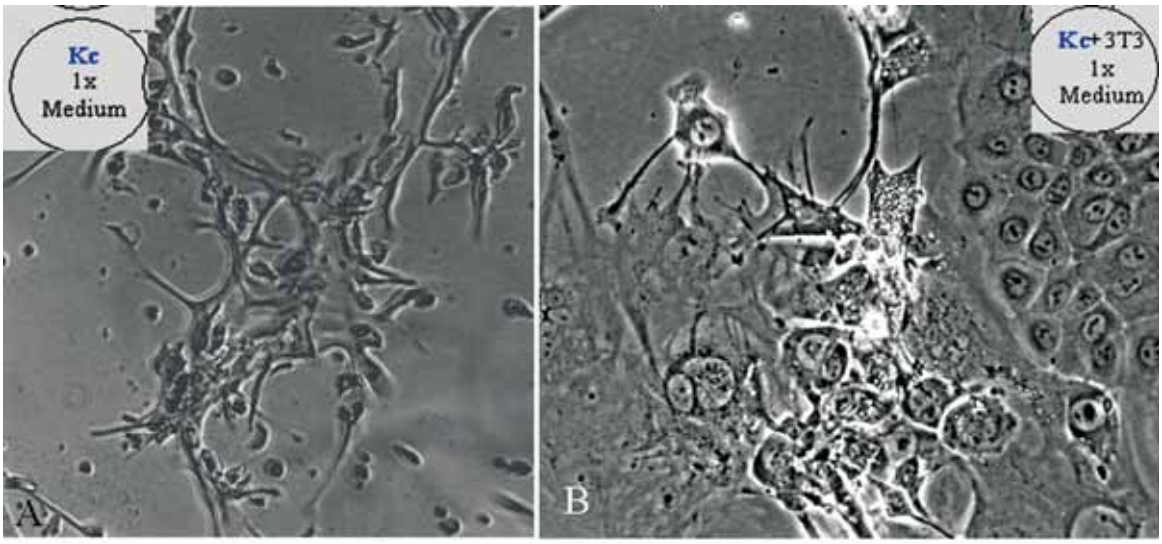


Fig. 9: Human epidermal Keratnocytes grown over plastic surface employing non-feeder culture (A) and Rheinwald-Green feeder dependant culture systems (B)

3. CELL CULTURE CONTAMINATION WITH MYCOPLASMA IN BASIC AND APPLIED BIOMEDICAL RESEARCH

(This is an ICMR funded SRF project. The SRF has been awarded Ph.D from Jamia Milia Islamia).

Scientific Staff	:	Dr. L.K. Yerneni, Ashok Kumar
Technical Staff	:	Bijender
Duration	:	2005-2008

Aims, Objectives and Background

Mycoplasma contamination in cell culture is a serious setback for the cell-culturist. The experiments undertaken using contaminated cell cultures are known to yield unreliable or false results due to various morphological, biochemical and genetic effects. Several international surveys revealed incidences of *Mycoplasma* contamination in cell cultures to range from 15 to 80%. There has been a lack of awareness about *Mycoplasma* contamination among cell culturists in our country and its significance in basic and applied biomedical research. Out of a vast array of methods for detecting *Mycoplasma* in cell culture, the cytological methods directly demonstrate the contaminating organism present in association with the cultured cells. It is now aimed to identify a simple yet dependable and quantifiable protocol for routine application in the detection of mycoplasmas in cell culture laboratories that employ human and other mammalian cell cultures.

Work done during the period

The cell cultures (primary and continuous) collected for the study from 21 Tissue Culture laboratories of various institutes situated in Delhi/outside Delhi were subjected to nested PCR using a detection kit from ATCC and the results were compared with cytological methods.

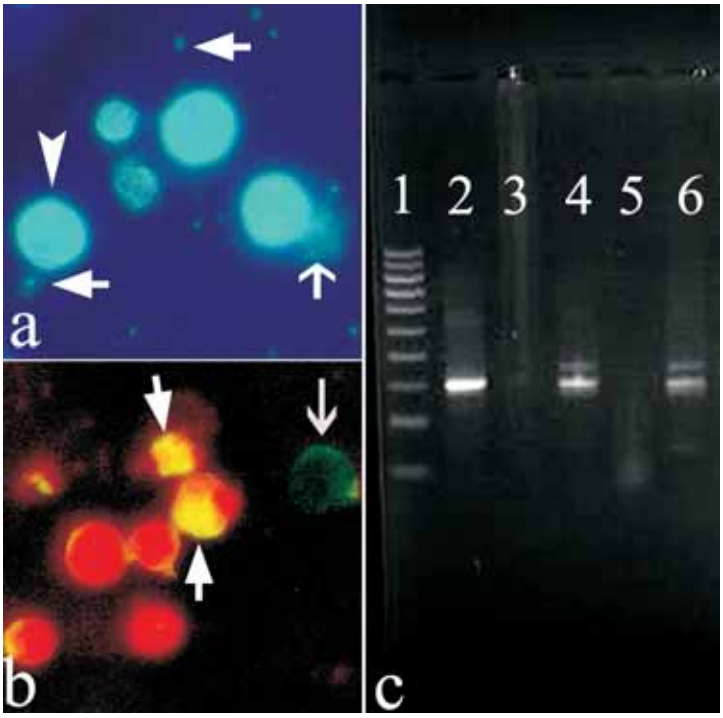


Fig. 1: The false negative and false positive mycoplasma contamination as demonstrated by nested PCR amplification.

a. DNA staining by Hoechst 33258 on a cell culture showing extensive mycoplasma contamination visible as discrete particles (solid arrows) or dense particulate matter (open arrow) in between cultured cell nuclei with blue green fluorescence (arrow head), Magnification 350X.

b. The Immunofluorescent assay performed on the same cell culture as in fig.a revealing the contamination in bright yellow-green fluorescence (solid arrow) over the red stained nuclei of cultured cells and also as yellow-green fluorescent particles lined up around a disintegrated cell periphery (open arrow) Magnification 350X.

c. The agarose gel electrophoretic separation of bands of 2nd stage nested PCR amplification products as visualized under UV trans-illuminator: - DNA ladder, lane 1; DNA of M. pirum, lane 2; template from the same cell culture as in Figures a & b showing no visible band indicating false negative status, lane 3; template from IFA and Hoechst positive cell culture with obvious bands showing a true positive result, lane 4; no template, lane 5 and template from IFA and Hoechst negative culture revealing clear bands representing a false positive result, lane 6.

The ATCC nested-PCR analysis revealed 64 cell cultures (83.1%) to be positive and 13 (16.9%) cell cultures negative for *Mycoplasma* contamination out of 77 cell cultures tested while, only 18 (23.4%) of the cell cultures were uniformly positive for *Mycoplasma* contamination by both Hoechst and IFA. The Hoechst staining of *Mycoplasma* contaminated cell cultures showed *Mycoplasma* as bright blue-green fluorescent particulate matter around the stained cell nuclei (Fig. 1a), while, the IFA preparations yielded a dual color presentation with *Mycoplasma* contamination in yellow-green fluorescence around the bright red cultured cells (Fig. 1b).

The 46 cultures showing negative result uniformly by Hoechst and IFA, revealed positive *Mycoplasma* contamination status by nested PCR and interestingly, one cell culture with highly convincing *Mycoplasma* positive status as evidenced by both Hoechst and IFA showed no demonstrable band on agarose gel after PCR amplification (Fig. 1c).

The non-specific amplification by double-step nested PCR methods could be due to amplification of intergenic spacer region between 16S and 23S rRNA genes as target sequence that have been widely used as targets to detect and identify many different types of bacteria and may not completely be specific for mollicutes resulting in false results.

Conclusions

In case double-step nested PCR approaches are adopted for screening of cell cultures, the tandem use of cytological tests of DNA staining and immunofluorescent assay is more practicable in evaluating and proper interpretation of PCR based results. On the whole, one should keep in mind that the primary goal in any *Mycoplasma* contamination should be to screen the cultures for contamination but not to specify the contaminant.

4. APPLICATION OF HUMAN EPIDERMAL SHEETS CULTURED FROM AUTOLOGOUS EPIDERMAL STEM CELLS IN BURNS PATIENTS IN PHASED MANNER

Scientific Staff	:	Dr. L.K. Yerneni
Technical Staff	:	Bijender
Duration	:	2005-2010

Aims, Objectives and Background

The clinical application of *ex-vivo* expanded human keratinocytes using the basic feeder cell based technique with our own in-house innovative research inputs to form Cultured Epidermal Autografts (CEA), requires development and practice of safer application protocol. Since, cell culture based therapeutic technologies are relatively new and the regulatory requirements towards such cell therapies vary from country to country, there is a need to design a cGMP compliant Clean Room Facility for producing clinical-grade graft material to undertake phased clinical application with CEA in burns patients. The designing of Clean Room is a highly specialized area and needs to be totally customized to the process of producing CEA and is hence undertaken through continuous interaction with experts in the area and visiting other similar clean facilities.

Work-done during the period

Designing of a class 10,000 cGMP clean room facility along with technical specifications was formulated for floating a tender, technical bids were scrutinized through a Technical Committee and the specifications were modified and frozen. In the meantime, a novel enclosed *ex-vivo* cellular therapy system from a US-based firm was evaluated as an alternative to the conventional Clean Room approach. This novel design appeared to be safer, compliant with current Good Tissue Practices (cGTP) of USFDA and requires lesser space and fewer personnel to maintain than the conventional system and presented to Pre-SAC for approval. Simultaneously, extra efforts as suggested earlier by SAC are also being made to undertake phased clinical trial with CEA through transfer of the developed technology to industry and the subject is being pursued with National Research Development Council (NRDC), New Delhi through IPR division of ICMR.

ENVIRONMENTAL TOXICOLOGY

ENVIRONMENTAL TOXICOLOGY

1. HEALTH HAZARDS OF PHTHALATES VIS-À-VIS IDIOPATHIC MALE INFERTILITY

Scientific Staff	:	Dr. Arun Kumar Jain, Rashmi Tomar
Collaborators	:	Dr. Sunil Kumar, NIOH, Ahmedabad Dr. N. K. Mohanty, Safdarjang hospital
Technical Staff	:	Mrs. Asha Rani Srivastava, Mrs. Anita Bhatia Mr. Suresh, Mr. Manoj
Duration	:	2006-2009

Aims, Objectives and Background

Plastics have been recognized as one of the greatest discovery of the millennium becoming an integral part of our life. Nobody can just think life without plastics. Unveiled by Alexander Parkes in 1862 at the Great International Exhibition in London, these synthetic polymers have the capability of being moulded, extruded, cast into various shapes. The two most common plastics in the world, in terms of production volume are polyethylene and PVC. Both are used in medical devices, packaging, electrical insulation, construction industry, flooring, some auto components and consumer goods. PVC is the only major commercial plastic that contains up to 45 percent chlorine by weight which renders the raw PVC useless making it brittle and prone to degrade rapidly when exposed to UV light and necessitating addition of several other chemicals such as antioxidants, lubricants, pigments, flame retardants and plasticizers to the resin to achieve desirable qualities. Almost all such PVC additives are a health concern; but in terms of the quantities used, the most important additives are plasticizers.

Almost 90% of plasticizers belong to a family of industrial compounds called phthalates (PAEs) which are used for a variety of purposes, including personal care, paints, industrial plastics, building materials, food packaging, clothing, toys, blood bags, intravenous fluid bags and infusion sets, and other medical devices (Huber et al. 1996). They are also used in detergents, solvents, lubricating oils, fixatives, adhesives, printing inks, aerosols, antifoaming agent, and coatings for paper (NTP-CERHR, 2003). PAEs are not chemically bound to the plastic so they can be released from consumer products into the environment thus becoming ubiquitous

environmental contaminants. Over a period of time, these accumulate in body tissues, and have been shown to damage liver, lungs, and reproductive organs in lower mammals. Some of the most common phthalates are Persistent Organic Pollutants (POP) or POP-like compounds. In both wildlife and laboratory animals, these have been linked to a range of adverse reproductive effects, *viz*., reduced fertility, miscarriage, birth defects, abnormal sperm counts, and testicular damage.

Because of their ability to disrupt the hormonal balance and impair reproduction and development, these are considered highly hazardous to human health. In a recent study on human male volunteer, Koch and Angerer (2007) have reported that phthalates are present in human body at levels far higher than previously believed, especially in children. In some cases, the measurements even exceed the TDI (tolerable daily intake), the highest dose a person can take in on a daily basis over his or her entire lifespan without any harmful effects. However, reports on adverse effects of phthalates in humans have been conflicting. Thus this study has been taken up with a view to study correlation between ultrastructural changes in the sperm and levels of marker testicular enzymes in blood on one hand and concentration of phthalates and their metabolites in urine of infertile males on the other.

Work done during the period

A total of 133 patients, who came for infertility treatment to Urology OPD of Safdarjang hospital, New Delhi were prospectively enrolled for this study. Twenty one healthy fertile subjects with less than one year old child were enrolled as controls. Based on occupation, the patients as well as controls were divided into 3 groups *viz*.: High, medium and low risk.

High risk group: Workers occupationally exposed to phthalates by virtue of working in plastics industry / shop.

Medium risk group: Individuals exposed to phthalates due to extensive usage of plastics at the home or work.

Low risk group / Control: Individuals reporting minimum exposure to phthalates at home or work as having normal fertility.

About 9% patients and 9.5% controls worked in soap, cosmetics, print, paint or plastic industry (high risk category) while 68.5% patients and 71.5% controls belonged to low-risk category.

Immediately upon collection, the volume of the semen along with colour and pH was recorded. Immediately thereafter, a drop of semen was used to study the vitality, motility and sperm count. It was followed by liquefaction of the semen. Liquefaction time was noted. Another drop of semen was used to prepare smear for morphological examination and the remaining semen was stored at -20°C for remaining studies.

Semen analysis

Semen amount varied from 0.5 ml to 5 ml (average 2.0 ml) in patients in comparison to 1.0 to 3.5 ml (average 2.35) in controls. It was less than 2 ml in about one-third of patients. The pH in majority of patients as well as controls ranged from 7.5 to 8.0. However, about 36% patients and about 28% controls revealed pH less than 7.4. About 7% of patients also had alkaline pH of more than 8.0 while none of controls had such high pH. Colour of the semen was white or pale white in majority of samples while in about 10% of patients each, it was pale yellow and greyish white.

Semen viscosity was normal in all the control samples except one. In patients, only about 51% samples revealed normal viscosity while 17% had high and another 32% had low viscosity. Liquefaction time was less than one hour in majority of patients and all controls, however, samples of 6 patients recorded liquefaction time of more than 2 hours. All the controls showed presence of more than 30% viable sperms in the semen while in patients about 32% had no sperms and only 21% of the remaining showed more than 30% viable sperms.

Further, the sperm count was more than 20 million per millilitre of semen in all the controls. All these samples showed normal motility and morphology. In patient group, about 31% semen samples showed no sperms and analysis of remaining samples revealed normal sperm count in only 15% , normal motility in about 45% and normal morphology in about 70% of samples having sperms (Table 1).

Table 1: Semen characteristics (Sperm count, motility and morphology)

Group	Sperm Count			Sperm Motility			Sperm Morphology		
	Nil	<20	≥20	Nil	Abnormal	Normal	Nil	Abnormal	Normal
Patients	41	78	14	41	48	44	41	23	69
Controls	0	0	21	0	0	21	0	0	21

Blood serum was used for assay of testosterone, estradiol, acid phosphatase, glutamyl transpeptidase and lactate dehydrogenase.

Testosterone levels

The testosterone levels varied from 1.72 to 13.10 units with average being 5.06 units in the patient group. On the other hand, in control groups, it varied from 3.65 to 16.30 units with an average of 7.44 units. With the increasing potential of occupational phthalate exposure, there was a significant corresponding decline in the testosterone levels (Table 2). The testosterone levels also showed corresponding decline with sperm count and motility morphology.

Table 2: Relation between testosterone levels and phthalate exposure risk

	Testosterone Level		
Risk Level	Min	Max	Average
0	1.86	13.10	5.26
1	1.79	12.80	5.02
2	1.72	5.15	4.15
All	1.72	13.10	5.06
Controls	3.65	16.30	7.44

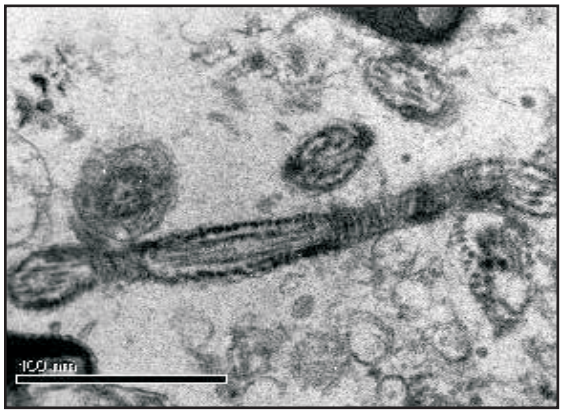
Estradiol levels

The estradiol levels varied from 0 to 137 units with average being 28.89 units in the patient group. On the other hand in control groups, it varied from 0 to 55 units with an average of 13.43 units. The estradiol levels were consistently higher in patient group in comparison with control group. While the data was highly skewed, the median levels provided more rationale 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 (Table 3). The testosterone levels also showed corresponding decline with sperm count and motility morphology.

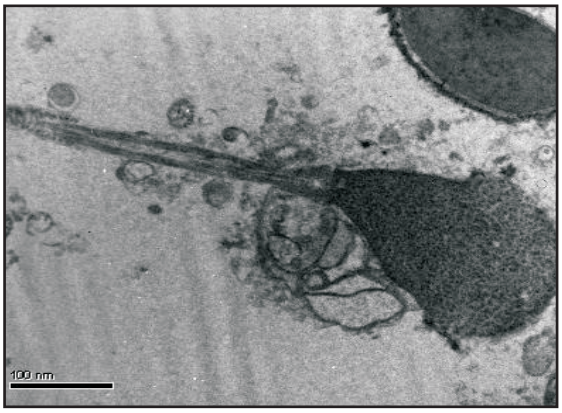
Table 3: Relation between estradiol levels and phthalate exposure risk

	Estradiol Level			
Risk Level	**Min**	**Max**	**Average**	**Median**
0	0.00	112.00	27.47	26.10
1	2.00	137.00	38.47	26.20
2	0.00	36.10	18.20	18.50
All	0.00	137	28.894	26.10
Controls	0.00	55.00	13.43	5.20

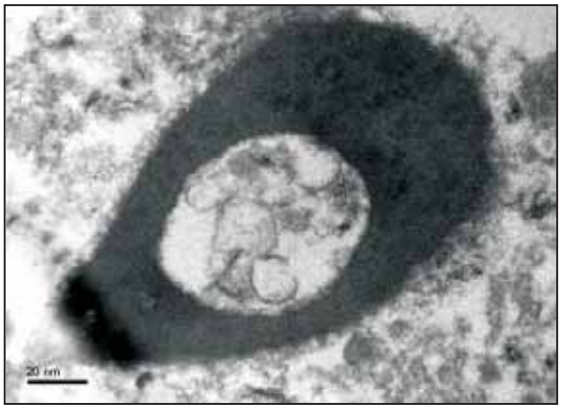
The ultrastructural studies revealed the altered micro-tubular arrangement in the tail of the sperms, presence of vacuoles in head cap, abnormalities in the acrosome, head shape and tail membranes, attachments of cytoplasmic body on sperm body and head of the infertile patients in comparison with fertile male.



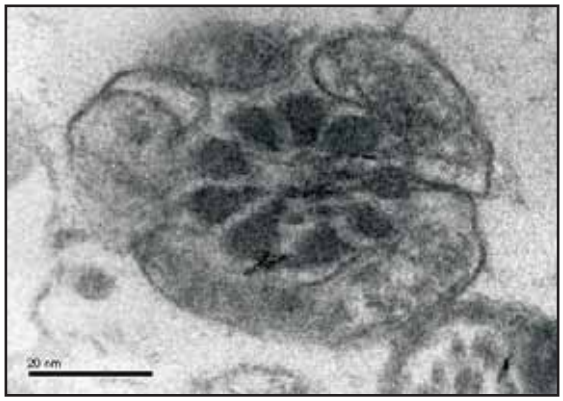
Hyperplasia of the fibrous sheath with portion of T.S. tail



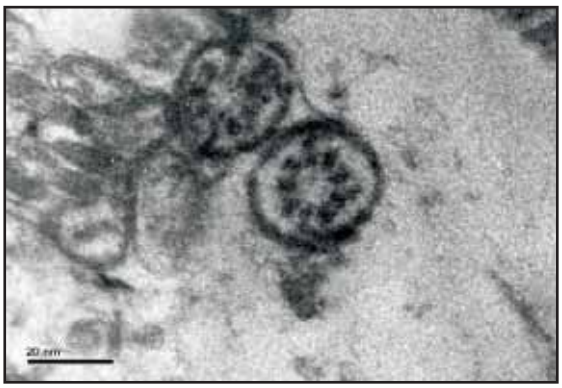
Early Immature spermatozoon with immature, Granular chromatin attached with abnormal tail



Oval shape multinuclear head with acrosome abnormalities



Central pair displaced & micro-tubular translocation



Two cross section of flagellum: Absence of central pair, Dynein arms are not properly visualized

2. ASSESSMENT OF PESTICIDE EXPOSURE IN TEA GARDEN WORKERS OF NORTH-EASTERN STATE OF INDIA (HEBM)

Scientific Staff	:	Dr. Arun Kumar Jain, Dr. S. Sriramachari, Dr. Madhu Bhatnagar
Collaborators	:	Dr. S. K. Sharma & Dr. A. M. Khan RMRC, Dibrugarh Dr. Deepa Borgohain, Assam Medical College, Dibrugarh Dr. Sudha Salhan, Safdarjang hospital
Technical Staff	:	Mr. Manoj
Duration	:	2008-2010

Aims, Objectives and Background

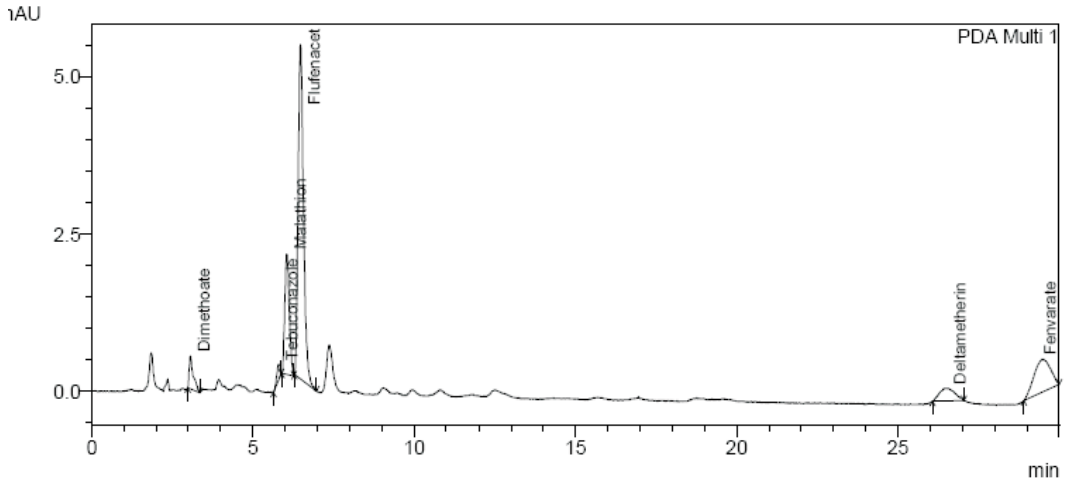
More than 50% of over 1.1 million workers in the labour intensive tea industry are women. The workers involved in activities such as pesticides spray, mixing and storing, rarely use any safeguard, take food without washing of hands and may even use jerry cans and bags (emptied after usage of pesticide) for storage of household food grain. An ICMR funded extramural 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.

Work done during the period

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, 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, 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 one 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.



PDA

ID#	Name	Ret. Time	Area	Height
1	Dimethoate	3.059	4595	524
2	Tebuconazole	5.798	1804	255
3	Malathion	6.043	18719	1915
4	Flufenacet	6.470	61166	5308
5	Deltamethrin	26.494	6585	202
6	Fenvarate	29.503	18353	509

Fig. 1 : Mixed Standard

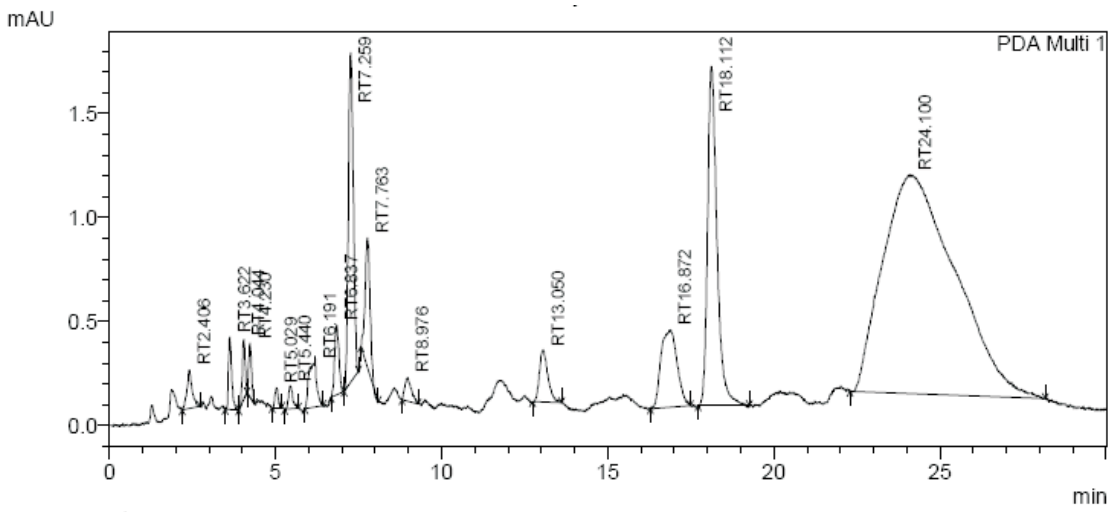


Fig. 2 : Chromatogram of placental tissue extract showing peaks of endosulfan (RT 13.0 5 & 16.8)

3. DYNAMICS OF ULTRA-STRUCTURAL AND IMMUNOLOGICAL EVENTS IN RESPONSE TO TREATMENT IN DIFFERENT FORMS OF PSORIASIS

Scientific Staff	:	Dr. Arun Kumar Jain, Dr. Usha Agrawal, Dr. Avninder Pal Singh
Collaborators	:	Dr. V. Ramesh, Safdarjang hospital
Technical Staff	:	Mrs. Asha Rani Srivastava, Mrs. Anita Bhatia, Mr. Suresh
Duration	:	2006-2009

Aims, Objectives and Background

Psoriasis is a complex chronic inflammatory condition of skin characterized by red, flaky, adherent silvery white scales or plaques on the elbows, knees, scalp, lower back, face, palms, and soles or anywhere on the body. Although rarely fatal, the disease worsens the quality of life and may lead to hospitalization in severe cases. It is a multifactorial skin disease of controversial aetiology that affects about 2% of world population with incidence varying among different ethnic groups (Raychaudhuri and Farber 2001). According to International Federation of Psoriasis Associations, more than 125 million people suffer from psoriasis and about 20%

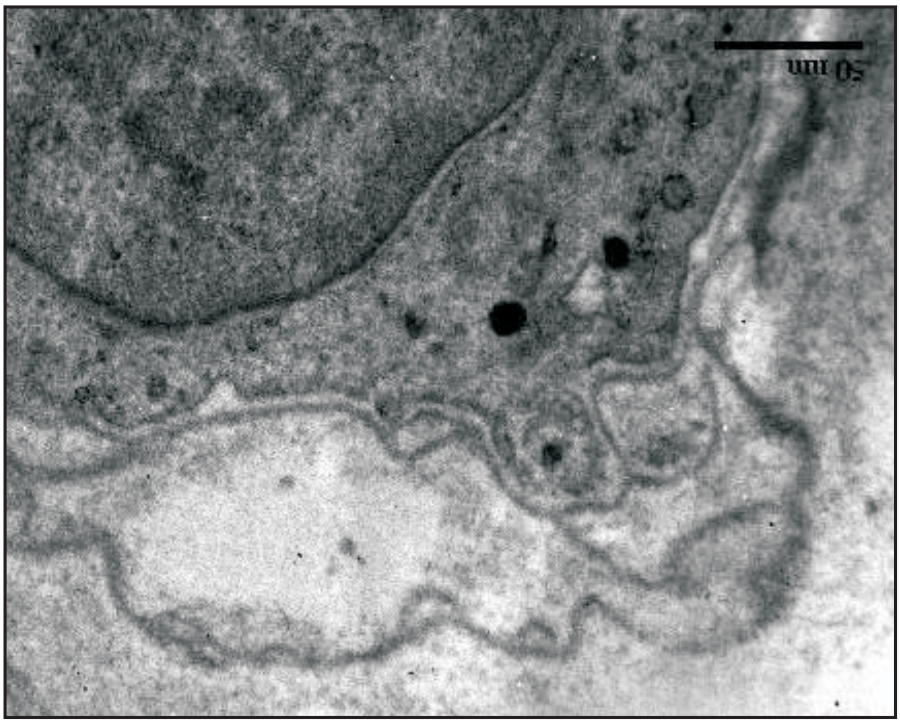
of them are in India. It is considered to be a disease of countries with cold weather. Still in terms of total number of patients, India ranks second only to China. It is a socially crippling disease badly affecting the quality of life and resulting in poor self-image, psychosexual distress, depression and social isolation. Due to absence of any experimental model and any definitive known cause of the disease, complete cure has so far not been discovered for 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. Although T-cell infiltration and keratinocyte hyper-proliferation are said to be key changes in the pathogenesis, over the years researchers have identified several ultrastructural, genetic and immunological factors which may play a role in the pathogenesis of disease still they have failed to establish a connecting link between all the factors.

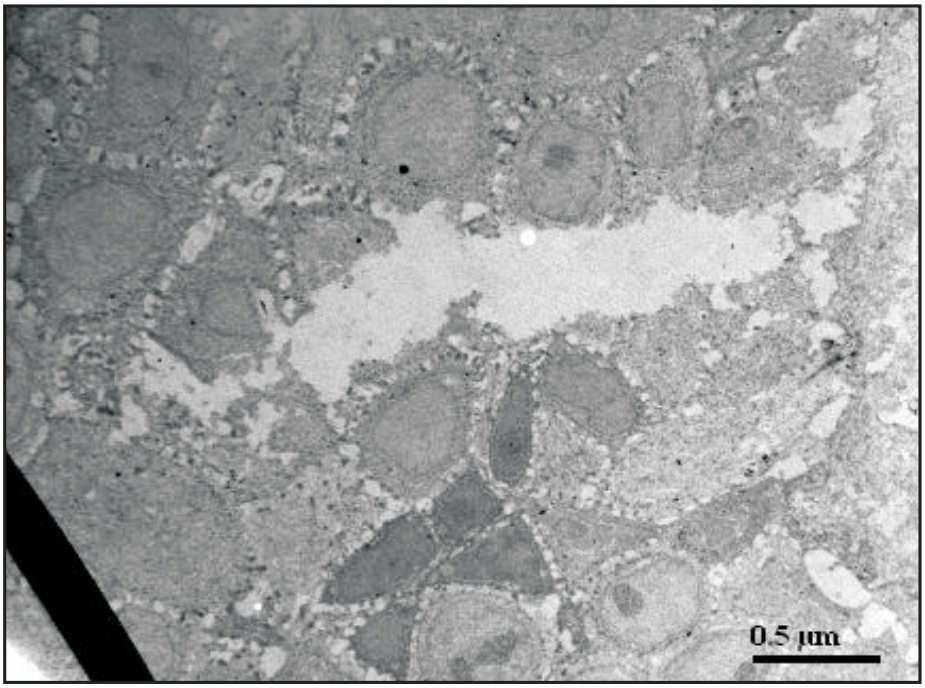
This study has been planned to investigate ultra-structural features of different type of psoriasis before and after methotrexate therapy.

Work done during the period

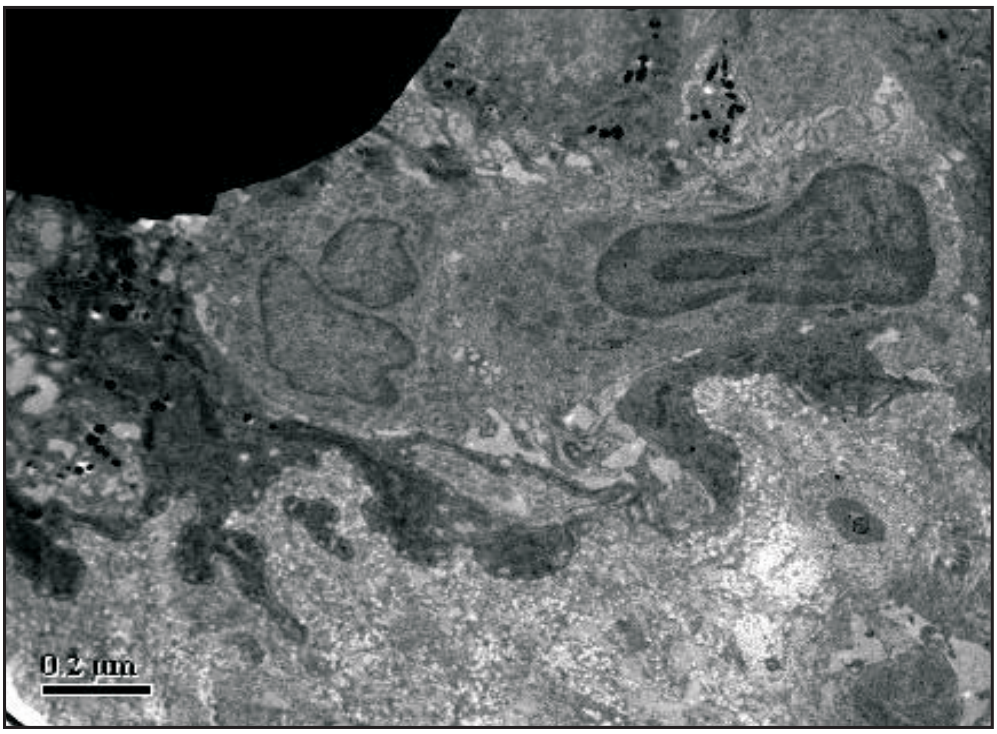
During the period under report, 30 patients were enrolled for the study. They were clinically examined and P.A.S.I. (Psoriasis Area Severity Index) score was calculated. 3 mm skin punch biopsies were taken from the margin of the lesional skin of the patient and uninvolved normal skin of the patient as control. The EM study revealed presence of neutrophils and lymphocytes 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' cells were regularly found which were confirmed by the presence of Birbeck granule. Dividing keratinocytes were observed in the lower layer of the psoriatic epidermis. The ultra-structural changes such as reduction of tonofilaments, dilatation of intercellular space, and interruption in basal lamina were recovered on methotrexate treatment resulting in normalization of the electron microscopic picture of psoriasis. The study is being continued.



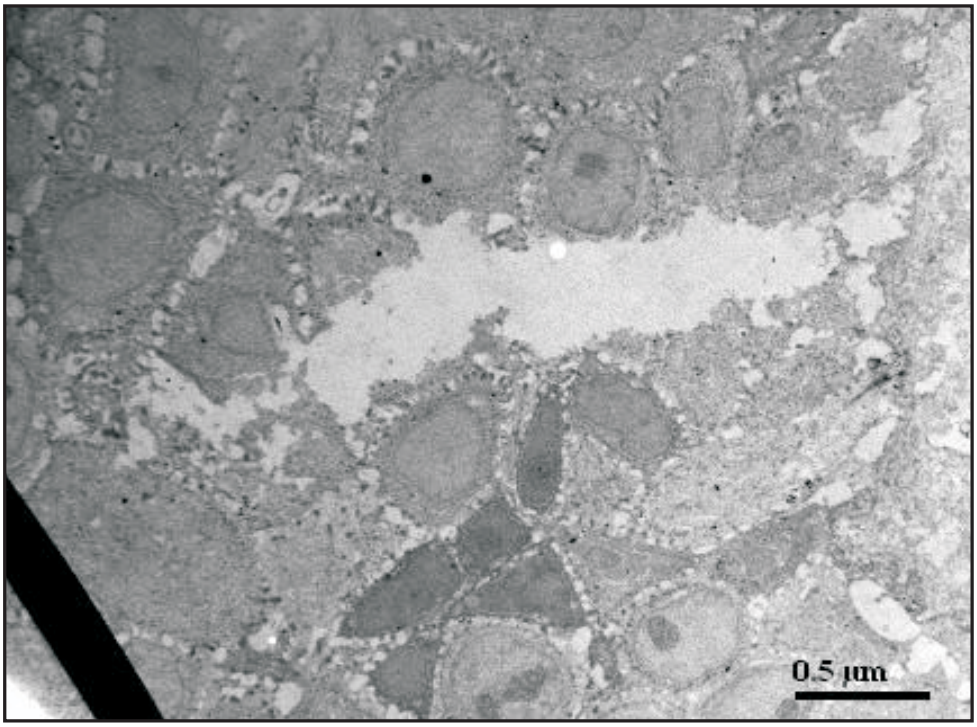
Electron Micrograph of dermo-epidermal junction in a patient from psoriasis shows re-duplication of basal lamina



Electron Micrograph of a Psoriatic Skin showing split in the epidermal layer.



Electron micrograph shows movement of inflammatory cells from dermis to epidermis in psoriatic skin.



Electron Micrograph of a sample from psoriatic skin showing a melanocyte that has been pushed to higher layers of epidermis.

ACADEMIC ACTIVITIES

SCIENTIFIC ACTIVITIES / SEMINARS / WORKSHOPS AT INSTITUTE OF PATHOLOGY

1. A **three day workshop** was organized at IOP from 16th -18th April 2007 on **'Microarray Technology'**.

2. **7th Smt. Pushpa Sriramachari Foundation Day Oration** by **Dr. MG Deo, Prof. Emeritus, National Academy of Medical Sciences** on **"Novel approaches in Medical and Biomedical Education and Research"** at Institute of Pathology on 1st May 2007.

3. **WHO workshops on Basic & Advanced Level Technicians Training Program** conducted from 16th April - 15th May 2007.

4. **DNB, six monthly appraisal** was conducted on 6th -7th August, 2007 with **Dr. Tejinder Singh**, Professor, MAMC, New Delhi as a local Appraiser.

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

6. **Breast Group Meeting** was held on 10th Aug. 2007 with invited guest lectures by **Dr. (Mrs.) Hoda**. The topics were :-
   1. Liquid based preparations
   2. Current issues in Diagnostic Breast Pathology
   3. Surgical Pathology of Breast

7. **Pre-Sac Meeting** on Tumor Biology/ Cell Biology was held on 2nd Nov. 2007 at IOP.

8. **Pre-Sac Meeting** on Infectious Diseases / Environmental Biology was held on 12th Nov. 2007 at IOP.

9. **SAC meeting** of IOP was held on 4th Dec. 2007 at National Institute of Immunology, New Delhi.

10. **Dr. WA Phillips**, Assoc. Prof. of Centre for Cancer Genomics & Peter MacCallum Centre, Australia delivered a lecture on **'Identfication of a high frequency of PIK3CA mutations in breast and ovarian cancer'** at IOP on 17th Dec. 2007.

11. **Hindi Day** was celebrated at IOP on 11th Jan. 2008. Lecture was given by **Mr. Dinesh Tripathy**.

12. A Guest lecture was given by **Dr. Ranju Ralhan**, Professor, Department of Biochemistry, AIIMS on 16th Jan. 2008. The topic of talk was "**Discovery of novel cancer biomarkers and drug targets using proteomics"**.

13. A Guest lecture was given by **Dr. Katrin Kuhls**, Charite University, Institute of Microbiology & Hygiene, Berlin, Germany on 24th Jan. 2008. The topic of talk was **"Population genetics studies of *Leishmania donovani* from Indian subcontinent using multilocus microsatellite typing".**

14. A Guest lecture was given by **Dr. Gopal Kundu**, National Science Centre, Pune on 11th Feb. 2008. The topic of talk was **"Significance of osteopenin in breast cancer".**

15. A presentation was given by **Labmate-India** on 20th March 2008. The topic of presentation was **"Denaturing High Performance Liquid Chromatography (DHPLC)".**

16. **8th 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.

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

18. 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.

19. **Independence Day** was celebrated at IOP on 14th Aug. 2008.

20. 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.

21. 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.

22. 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.

23. 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.

24. 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.

25. 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.

26. 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.

27. **Pre- Sac Meeting** on Tumor Biology/ Cell Biology/ Infectious Diseases / Environmental Biology was held on 17th March and 20th March 2009 at IOP.

28. 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.

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

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 received **Basanti Devi Amir Chand Award** for the year 2006 conferred by Indian Council of Medical Research in July 2007.

3. Dr. Poonam Salotra received **Prof. BK Aikat Oration Award** for the year 2004 conferred by Indian Council of Medical Research in July 2007.

4. Dr. Poonam Salotra elected **Fellow of the National Academy of Sciences, India** during 2008.

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

6. 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 17th –20th January, 2009.

7. Ms. Mishi, SRF and Ph.D. student won **Best Poster award** on "Assessment of breast cancer risk: Genotype polymorphism in estrogen synthesizing and metabolizing genes and their contribution in breast cancer susceptibility" at 27th Annual Convention of Indian Association for Cancer Research - Networking Research to Applications & International Symposium on Frontiers in Functional Genomics at Ahmedabad from 6th – 9th February 2008.

8. Ms. Abha Soni, SRF and Ph.D. student won **Best Poster Award** in "XXXIII Annual Conference of the ISHG and International Symposium on Genetics Revisited: The Genomics and Proteomics Advantage" held in Visakhapatnam from 11th to 13th Feb. 2008 on poster entitled "What Androgen Receptor CAG repeats polymorphism and p53 mutations/polymorphisms have to do with prostate cancer risk and progression?"

9. 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.

10. 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. Poonam Salotra** received **Bill and Melinda Gates Travel award** for participating in Keystone symposium in California, USA in Jan 2007.

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

3. **Dr. AK Mishra** received the **'World Bank-Travel Grant'** to participate in the "56th International Statistical Institute Conference" held at Lisbon (Portugal) from 22nd - 29th August, 2007.

4. **Indranil Chattopadhyay,** Ph.D. Scholar awarded **ICRET fellowship** of **UICC**.

5. **Apurb Rashmi Bhengraj** was awarded scholarship for participation in the Microbicides 2008 Conference.

TECHNOLOGIES FOR COMMERCIALIZATION

1. Development of serovar specific monoclonal antibody to *Chlamydia trachomatis* to BCIL.

PATENTS

Patents Filed for:

1. Development of primary cervical epithelial cell line from cervical lavage

2. Development of Dot-blot assay for prognosis of sequelae to *Chlamydia trachomatis* infection in women using chlamydial heat shock protein 60.

SCIENTISTS ATTENDED CONFERENCE / WORKSHOP / FELLOWSHIP ABROAD

1. **Dr. Sunita Saxena** attended the NCRI Cancer Conference held at International Convention Centre in Birmingham, UK from 30th September to 3rd October 2007 and presented paper entitled "Gene expression profile of esophageal cancer in North East India by cDNA microarray analysis".

2. **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 12th -17th June 2008.

3. **Dr. Aruna Singh** attended 'World Congress On International Union Against Sexually Transmitted Infections and International Society For STD Research' at Washington Seattle USA, from 29th July to 2nd August 2007.

4. **Dr. Aruna Singh** attended Chlamydia Euro Meeting at Aarhus, Denmark from 1st July - 4th July 2008.

5. **Dr. Poonam Salotra** visited lab. of Dr. Emanuela Handman at The Walter Eliza Hall Institute of Medical Research, Royal Melbourne Hospital, Australia for 2 weeks in Feb. 2007 under ICMR International Fellowship for Senior Indian Biomedical Scientists.

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

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

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

9. **Dr. Poonam Salotra** participated in Keystone symposium in California, USA in Jan. 2007. Presented work entitled "Challenges in the treatment of visceral leishmaniasis: Potential of marine natural products as alternative drug candidates".

10. **Dr. VP Singh** presented Poster at 'TraceSpec2007: 11th Workshop on Progress in Analytical Methodologies for Trace Metal Speciation" jointly organized by the University of Munster Institute of Inorganic and Analytical Chemistry, the European Institute for Speciation Analysis and the International Association of Environmental Analytical Chemistry, Munster, Germany during 4th –7th Sept. 2007.

11. **Dr. AK Mishra** attended '56th International Statistical Institute Conference' at Lisbon, Portugal from 22nd - 29th August 2007 and presented the research paper entitled "Some Epidemiological Models for the Parasitic Infections: Comparison between Traditional and Hierarchical Logistic Regression Methods".

12. **Indranil Chaterjee, Ph.D. student** and SRF trained on Copy number analysis at Peter MacCallum Cancer Centre, Melboune, Australia in 2007.

13. **Dhiraj Kumar, Ph.D. 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 from 22nd – 26th March 2009.

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

EXTRAMURAL PROJECTS

NEW PROJECTS

2007-08

1. Study on gene expression and hyper-methylation profile in early onset breast cancer.
   Dr. Sunita Saxena, Dr. Sujala Kapur, Dr. BSA Raju - DBT (2008-2011).

2. Immunomolecular expression of prostaglandin receptors, cyclooxygenase & tumor necrosis factor-alpha in endometrial curettage tissue of *C. trachomatis* infected women during failed pregnancy.
   Dr. Sangita Rastogi – ICMR (2008-2011).

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

2008-09

1. Characterisation of host immune factors associated with progression of superficial TCC of bladder by microarray analysis.
   Dr. Sunita Saxena - ICMR (2009-2012).

2. 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).

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

4. 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).

COMPLETED PROJECTS

2007-08

1. Role of chlamydial heat shock proteins in pathogenesis of genital tract infection in women.
   Dr. Aruna Singh – DRDO (2003-2007)

2. Study of virulence genes in Indian strains of *Chlamydia trachomatis*.
   Dr. Aruna Singh – DBT (2005 - 2008)

3. Immune responses to *Chlamydia trachomatis* infection in spontaneous aborters.
   Dr. Sangita Rastogi – DRDO (2004-2007)

4. Cell culture contamination with *Mycoplasma* in basic and applied biomedical research
   Dr. L K Yerneni – ICMR (2005 - 2008)

2008-09

1. Comprehensive study of carcinoma oesophagus at northeast India - multidiscipline approach.
   Dr. Sunita Saxena, Dr. Sujala Kapur - ICMR Multicentric Task Force Project (2004-2008)

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)

ONGOING PROJECTS

2007-2009

1. Microsatelite instability in androgen receptor gene, p53gene polymorphisms/mutations and expression profile of mismatch repair genes in prostate cancer.
   Dr. Sunita Saxena - DST (2005-2009)

2. Effect of pesticide exposure in causation of cancer in north east India.
   Dr. Sunita Saxena, Dr. Sujala Kapur, Dr. Usha Agrawal, Collaborative, Multicentric, ICMR Task Force Project (2005-2010)

3. Role of tobacco use in causation of cancer in north east India.
   Dr. Sunita Saxena, Dr. Sujala Kapur, Dr. Usha Agrawal, Collaborative, Multicentric, ICMR Task Force Project (2005-2010)

4. Establishment and characterization of breast cancer cell lines from primary breast cancers.
   Dr. Sunita Saxena, Dr. Sujala Kapur, Dr. SA Raju Bagadi - ICMR Task Force Project (2007-2010)

5. Role of *Chlamydia pneumoniae* in Coronary Artery Disease (CAD) patients.
   Dr. Aruna Singh – DST (2007-2010)

6. Effect of sex hormones on induction of immunity by dendritic cells in female reproductive tract during *Chlamydia trachomatis* infection.
   Dr. Aruna Singh – DBT (Indo-US CRRH) (2005 - 2009)

7. Evaluation of host determinants involved in pathogenesis of Kala-azar and post kala-azar dermal leishmaniasis using cDNA array.
   Dr. Poonam Salotra – DRDO (2006 - 2009)

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

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

10. Investigations into the utility of a patented syntheic therma-reversible hydrogel polymer as supportive matrix towards the development of 3-D composite skin for application in wound healing and other dermatological disorders.
Dr. L K Yerneni – ICMR (2007-2010)

PUBLICATIONS

In Journals

(2007-08)

1. Chintamani, Kulshreshtha Pranjal, Sugandhi Nidhi, **Bansal Anju**, Bhatnagar Dinesh, **Saxena Sunita**. Is an aggressive approach justified in the management of an aggressive cancer- The squamous cell carcinoma of thyroid? ***International Seminars in Surgical Oncology***, 4: 8 doi:10.1186/1477-7800-4-8, 2007.

2. Chintamani, Khandelwal Rohan, Mittal Aliza, Saijanani Sai, Tuteja Amita, **Bansal Anju**, Bhatnagar Dinesh, **Saxena Sunita**. Qualitative and quantitative dermatoglyphic traits in patients with breast cancer: A prospective clinical study. ***BMC Cancer***, 7: 44, 2007.

3. Chintamani, Singhal Vinay, **Bansal Anju**, Bhatnagar Dinesh, **Saxena Sunita**. Isolated colostomy site recurrence in rectal cancer-Two cases with review of literature. ***World Journal of Surgical Oncology***, 5: 52, 2007.

4. Kumar A, Singh S, Salhan S, **Mittal A.** Evaluation of developed species specific monoclonal antibody for detecting *Chlamydia trachomatis* infections in endocervical specimens from female patients. ***Hybridoma***, 26: 333-337, 2007.

5. Jha HC, Vardhan H, Gupta R, Prasad J, Verma R, **Mittal A.** *Chlamydia pneumoniae* infection in coronary artery disease patients in India. ***Nature India,*** 3: 1, 2007.

6. Kumar R, Bumb RA, Ansari NA, Mehta RD, **Salotra P**. Cutaneous leishmaniasis caused by *Leishmania tropica* in Bikaner, India: Parasite identification and characterization using molecular and immunologic tools. ***Am J Trop Med Hyg***, 76(5): 896-901, 2007.

7. Srividya G, Duncan R, Sharma P, Raju BV, Nakhasi HL, **Salotra P.** Transcriptome analysis during the process of *in vitro* differentiation of *Leishmania donovani* using genomic microarrays. ***Parasitology***, 134: 1527-39, 2007.

8. Ramesh V, Singh R, **Salotra P**. Short communication: Post-kala-azar dermal leishmaniasis- An appraisal. ***Trop Med Int Health***, 12: 848-51, 2007.

9. Ramesh V, Kumar J, **Salotra P**. An unusual presentation of post-kala-azar dermal leishmaniasis. ***Trop Doct***, 37(3): 172-3, 2007.

10. Subba Raju BV, Singh R, Sreenivas G, Singh S, **Salotra P**. Genetic fingerprinting and identification of differentially expressed genes in isolates of *Leishmania donovani* from Indian patients of post-kala-azar dermal leishmaniasis. ***Parasitology***, 135: 23-32, 2008.

11. 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.

12. Sethuraman G, Sharma VK, **Salotra P**. Indian mucosal leishmaniasis due to *Leishmania donovani* infection. ***N Engl J Med***, 358: 313-5, 2008.

13. Kumar R, Ansari NA, Singh A, Ramesh V, **Salotra P**. Cutaneous leishmaniasis in Nepal : *Leishmania major* is a cause. ***Trans Roy Soc Trop Med Hyg***, 102: 202-3, 2008.

14. Kumar D, Srividya G, Verma S, Singh R, Negi NS, Fragaki K, Kubar J, **Salotra P**. Presence of anti-Lepp12 antibody: A marker for diagnostic and prognostic evaluation of visceral Leishmaniasis. ***Trans Roy Soc Trop Med Hyg***, 102: 167-71, 2008.

15. Bhushan B, Ahuja D, **Verma S**, Saluja S, Siddiqui S, **Kapur S.** Relation of cell viability and apoptosis with clinical remission following induction chemotherapy in ALL and AML. ***Journal of Experimental and Clinical Cancer Research***, 26(3): 313-321, 2007.

16. Sharma Monika, Sharma Sadhna, Roy Sugata, **Varma Saurabh**, Bose Mridula. Pulmonary epithelial cells are a source of interferon-γ in response to *Mycobacterium tuberculosis* infection. ***Immunology and Cell biology***, 85(3): 229-237, 2007.

17. Kumar Ashok, Ali, **Yerneni LK**. Effectiveness of *Mycoplasma* elimination reagent on Arif, a hybridoma cell line with lab. acquired extensive *Mycoplasma* contamination. ***Hybridoma***, 26 (2): 104-106, 2007**.**

18. **Avninder S**, Bhatnagar A, **Agrawal U, Saxena S**. Isolated splenic metastasis from colorectal mucinous carcinoma. ***Int J Gastrointes Cancer***, 37: 98-101, 2006.

19. Kumar R, Ansari NA, **Avninder S**, Ramesh V, **Salotra P**. Cutaneous leishmaniasis in Nepal: *Leishmania major* as a cause. ***Trans R Soc Trop Med Hyg,*** 102(2): 202-03, 2008.

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25. 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.

26. **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.

27. Negi Shivani, **Mishra AK**, **Bansal Anju**, Bhatnagar Amar, Bhatnagar Dinesh, Chintamani, **Saxena Sunita**. 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.

28. **Yerneni LK**. Bioengineering of CEA from adult epidermal stem cells. The Future perspective. *In*: Proceedings of Symposium on Therapeutic Potential Of Stem Cells, Nichi-In center for Regenerative Medicine, 27th October 2007, Chennai, 2007.

29. **Yerneni LK**, Kumar A. (2007). Adult Epidermal Stem Cells: Isolation, Maintenance and Differentiation. *In*: Proceedings of Training course entitled "Basic Techniques in Stem Cell Biology: Isolation, Maintenance and differentiation" ADNAT, CCMB, Hyderabad 25th February to 10th March 2008.

PARTICIPATION IN SCIENTIFIC ACTIVITIES

Dr. Sunita Saxena

1. Attended the NCRI Cancer Conference held at International Convention Centre in Birmingham, UK from 30th September - 3rd October 2007 and presented paper (oral and poster) entitled "Gene expression profile of esophageal cancer in North East India by cDNA microarray analysis"

2. Invited as a guest speaker in CME entitled "Ancillary Techniques in Anatomic Pathology from Digital Imaging to Confocal Imaging and Laser Microdissection" at 56th Annual Conference of the Indian Association of Pathologists and Microbiologists [APCON 2007] held at PGIMER, Chandigarh from 26th -29th November 2007.

3. Scientific Advisory Committee Meeting of Institute of Pathology was held on 4th December 2007 at the National Institute of Immunology, New Delhi.

4. Nominated by WHO for the meeting on "Research Policy and Management of Risk in Life Science Research for Global Health Security" held on 10th - 12th December 2007 at Bangkok, Thailand.

5. Viva of the six Pre-Ph.D. students of the off-campus programme of BITS, Pilani was conducted on 21st December 2007 at the Institute. The viva was conducted by Prof. Ravi Prakash, Dean, Research and Consultancy & Profesor of Mechanical Enginerring BITS, Pilani and Prof. Nirupama Prakash, Co-ordinator, Hospital & Health System Management, BITS, Pilani.

6. Attended lecture by Nobel Laureate David Baltimore on "New Avenues to Health" held at Teen Murti Auditorium, New Delhi on 14th January 2008.

7. Attended Annual Meeting of Indian Association of Cancer Research held at Ahmedabad on 7th – 8th February 2008.

8. Attended Project Review Committee Meeting of North-East in the NCD Division of I.C.M.R. held at ICMR, New Delhi on 12th February 2008.

9. Invited as external examiner for the Ph.D. students at the All Indian Institute of Medical Sciences, New Delhi on 12th February 2008.

10. Conducted inspection of the Department of Pathology, Rajshree Chhatrapati Shahu Maharaj Government Medical College, Kolhapur for renewal of DNB accreditation on 22nd February 2008.

11. Attended Project Review Committee Meeting of the BMS Division held at ICMR, New Delhi on 25th February 2008.

12. Attended Project Review Committee Meeting of the NCD Division held at ICMR, New Delhi on 3rd March 2008.

13. Attended a brain storming Meeting on Gene Therapy in Cancer held at ICMR, New Delhi on 4th March 2008.

14. Delivered a talk on "Breast cancer risk factors in north-east Indian women" in Breast Con-2008 held at Guwahati on 7th March 2008.

15. Chaired session on Non-communicable diseases during conference on "Show casing Science by Indian Women Scientist" held on 8th – 10th March, 2008 and delivered a talk on "Genetic profile of breast cancer in Indian women".

16. Attended the Scientific Advisory Committee meeting of the Institute of Cytology & Preventive Oncology held in Noida on 26th March, 2008.

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

18. 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.

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

20. 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".

21. 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.

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

23. 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.

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

25. 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.

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

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

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

29. 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".

30. Attended HUGO's 13th Human Genome Meeting during 27th – 30th September 2008 in Hyderabad.

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

32. 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.

33. Delivered a talk on 'Prognostic and predictive factors in cancer management' at Foundation Day Celebration & Workshop on Brachytherapy, Department of Radiotherapy at CSM Medical University, Lucknow on 14th December 2008.

34. 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.

35. 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 14th -15th Feb. 2009 organised by Department of Pathology, Amrita Institute of Medical Sciences, Kochi.

36. 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.

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

38. 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.

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

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

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

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

43. 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.

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

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

46. 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.

47. 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.

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

Dr. Aruna Singh

1. Invited speaker at Joint Working Group Committee Meeting on 'Indo-US Contraceptive Research & Reproductive Health' in Delhi on 19th September 2007. Title of talk was 'Effect of sex hormones on induction of immunity by dendritic cells in female genital tract during *Chlamydia* infection'.

2. Invited speaker during the 'Workshop on opportunities and limitations in developing diagnostics' jointly organized by Department of Biotechnology and Yashraj Biotechnology at Mumbai in Nov. 2007. The title of talk was ''Diagnosis of *Chlamydia in* India: Limitations and need for development of new technologies'.

3. Invited speaker at 'CME update on community acquired multidrug resistance infections' organized by Department of Microbiology, Safdarjung hospital on 18th Jan 2008. The title of talk was 'Emerging Resistance in *Chlamydia trachomatis* -Global Scenario'.

4. Invited speaker at Indo-Finnish meeting organized by DBT in Manesar from 23rd -24th Jan 2008. The topic of talk was 'Chlamydial Diagnosis: Biomarkers and new opportunities for rapid tests'.

5. Nominated to attend 'International Conference on Microbicides' organized by ICMR at Ashoka Hotel on 24th -27th Feb 2008.

6. 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'.

7. Invited to attend viva exam of 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.

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

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

10. 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".

11. 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".

12. 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, 2007-08.

2. Attended Meeting with the Collaborators from Northeast India at NIOH, Ahmedabad on 20th and 21st August 2007.

3. Attended Project Review meeting of Gastroenterology held at ICMR Headquarters, New Delhi on 23rd August 2007.

4. Attended 'Update In Surgical Pathology 2007 held at Tata Memorial Hospital, Mumbai on 31st August - 2nd September 2007.

5. Attended Meeting with the Collaborators from Northeast India held at Dr. B Barooah Cancer Institute, Guwahati on 18th September 2008.

6. Attended Meeting of Project Review Committee, Division of NCD, ICMR held at Guwahati on 27th - 28th December 2007.

7. Attended 'Molecular Medicine Update' on the Golden Jubilee year of the inception of Maulana Azad Medical College, New Delhi on 28th -29th March 2008.

8. Organised Workshop - "National Workshop on Microarray Technology" held from 16th - 18th April, 2007 at Institute of Pathology, New Delhi.

9. Invited Speaker 'Application of microarray technology in cancer biology' in "National Workshop on Microarray Technology" held from 16th -18th April, 2007 at Institute of Pathology, New Delhi.

10. Invited Speaker – 'Microarray Technology -Application of Gene Expression Profile in Pathology Practice' in Meeting of Indian Association of Pathologists and Microbiologists, Delhi Chapter, August 2007.

11. Examiner for M.Sc. Jamia Hamdard University, Hamdard Nagar, New Delhi, 29th November 2007.

12. Invited Speaker – 'Emerging role of microarray technology in cancer diagnosis and prognosis in molecular medicine update' on the Golden Jubilee year of the inception of Maulana Azad Medical College, New Delhi, 28th - 29th March 2008.

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

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

15. Attended Project Review meeting of Gastroenterology held at ICMR Headquarters, New Delhi, 2008.

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

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

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

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

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

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

Dr. Sangita Rastogi

1. Member, Selection Committee for Technical Assistant held at Institute of Pathology, New Delhi on 2nd April 2007.

2. 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.

3. Co-ordinated the annual inspection of the Institute Animal House by Dr. Ravindra Sharma, CPCSEA nominee on 31st July 2007.

4. Member, Seleciton Committee for Junior Research Fellow and Technical Assistant held at Institute of Pathology, New Delhi on 17th August 2007.

5. Attended the Launching Ceremony of Department of Health Research on 5th October 2007 at Hotel Imperial, New Delhi.

6. Attended the ICMR Annual Day Oration Lecture titled, 'Major trends for biomedical research in the 21st century: The view from NIH' delivered by Dr. Elias A. Zerhouni on 5th October 2007 at Hotel Imperial, New Delhi.

7. Compiled Performance budget document of IOP for onward submission to MOHFW in October 2007.

8. Examiner for M.Sc. Theory Examination of CCSU (Jan. 2008).

9. Participated in 51st All India Congress of Obstetrics and Gynecology on 2nd-5th February 2008 at Hotel Ashoka, New Delhi.

10. Member, Editorial Board, IOP Annual Report.

11. Teaching faculty & Examiner for off-campus pre-Ph.D. Program of BITS, Pilani and undertook a course on Technical Communication.

12. 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.

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

14. 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.

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

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

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

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

19. Participated in 25th Annual Conference of ISOPARB-2009 on 21st -22nd March 2009 at India Habitat Center, New Delhi.

Dr. Poonam Salotra

1. Participated in Ranbaxy Science Foundation Symposium on "Emerging Frontiers in Control of Cancer" at National Institute of Immunology, New Delhi on Jan. 13, 2008.

2. Attended the seminar at IOP on "Discovery of novel cancer biomarkers and drug targets using proteomics" - by Dr. Ranju Ralhan, Professor at AIIMS and Visiting Professor at York University, Toronto on 16th Jan. 2008.

3. Invited to be on Selection Committee for Research Associate interview at NII, New Delhi on 10th Jan. 2008.

4. Guided completion of PhD thesis work of 1 student and its submission to Jiwaji University in Sept. 07.

5. Appointed as examiner for PhD viva, at Jiwaji university, Gwalior on Dec. 26, 2007.

6. Co-ordinator of Academic Cell of Institute of Pathology, responsible for organizing the course work for PhD students.

7. Examiner and Co-ordinator for PhD qualifying examination at IOP for BITS, Pilani programme held in June 07 and Dec 2007.

8. Co-ordinator of Journal Club and weekly seminars at IOP.

9. Guided M Sc dissertation work of one student from Jiwaji University for 6 months from Jan 08.

10. Guided research work of 4 students registered for PhD with BITS Pilani.

11. Member of Editorial Committee of Institute News Letter.

12. Reviewer for several projects submitted for funding to ICMR, DBT and DST.

13. Participated in the symposium of Patel Chest Allumini organized by Department of Biochemistry, University of Delhi South Campus at SP Jain auditorium, July 2nd, 2008.

14. Organised a guest Lecture at Institute of Pathology by Dr Caryn Bern entitled "The epidemiology of Visceral Leishmaniasis and PKDL with a focus on recent studies from Bangladesh"on 4th September, 2008.

15. Attended seminar at IOP on "Curcumin, piperine and curcumin dipiperoyl in Breast cancer prevention"- by Dr Madhuri Kakarala, from University of Michigen, MI, USA, November 12th, 2008.

16. Participated in meeting "India and Germany –Strategic Partners for innovation" held at India Habitat Centre, New Delhi on 9th September 2008.

17. Attended seminar at IOP on "Novel strategies in Vaccine development"- by Dr FO Eko, from Morehouse school of Medicine, Atlanta, Geogia, USA on 24th November, 2008.

18. Examiner for PhD thesis on *Leishmania* titled "Molecular and Immunological Aspects in Leishmaniasis" submitted to Banaras Hindu University in April, 2008.Visited BHU to conduct Viva Voice Examination on 27th Nov. 2008.

19. Visited the Parasitology Lab in CDRI, Lucknow and delivered a talk on Post Kala Azar dermal Leishmaniasis in India on 8th December 2008.

20. 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.

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

22. Participated in the Kick-off Meeting and Workshop at Carros, France under the new collaborative project funded by European Commission entitled "Pre-clinical studies of a PSA-based human vaccine candidate targeting visceral, cutaneous and mucocutaneous leishmaniasis and development of the associated procedures for further clinIcal trials" (RAPSODI) in Jan. 2009.

23. Participated in the Kick-off Meeting and workshop at BHU, Varanasi under the new collaborative project funded by European Commission entitled "New tools for monitoring drug resistance and treatment response in visceral leishmaniasis in the Indian subcontinent" (KALADRUG) in Feb. 2009.

24. Participated in the Molecular Immunology Forum (MIF) at Alibagh, Mumbai in March 2009.

25. Appointed external examiner for thesis evaluation of Ph.D work at CDRI, Lucknow in Dec. 2008.

26. Co-ordinator of Academic Cell of Institute of Pathology, responsible for organizing the course work for PhD students.

27. Guided research work of 6 students registered for PhD with BITS Pilani.

28. Reviewer for several manuscripts submitted to international journals.

29. Appointed Associate Editor of a BMC journal in 2009.

Dr. AK Jain

1. Off campus Ph.D. Programme of BITS, Pilani. Course G644 : Development and use of computer software.

2. Coordinator, WHO In-country Fellowship Training Program (Basic and Advanced level training course for Technicians) for the Biennium 2006-07 from 16th April to 15th May 2007.

3. Coordinator, WHO In-country Fellowship training program for Pathologists for the Biennium 2006-07 from 4th June to 3rd July 2007.

4. Third Medical Development Congress 2007 on "Can traditional wisdom be super modern" at Assocham House, Prithvi Raj Road, New Delhi on Sept. 15-16, 2007.

5. Launching ceremony of the Department of Health Research on 5th Oct 2007 at Hotel Imperial, New Delhi.

6. Inauguration of Vardhman Mahavir Medical College Building by Hon'ble Vice President of India on 20th Nov. 2007 at Safdarjang Hospital, New Delhi.

7. Member, Steering Committee for organization of National Conference on Electron Microscopy and Allied Fields held at University of Delhi, Delhi from 26-28 November 2007.

8. Invited to chair a scientific session at XXIX Annual Meeting of EMSI and National Conference on Electron Microscopy and Allied Fields held at University of Delhi, Delhi from 26th –28th November 2007.

9. XXIX Annual Meeting of EMSI and National Conference on Electron Microscopy and Allied Fields held at University of Delhi, Delhi from 26-28 November, 2007.

10. Invited talk on 'Relevance of Electron Microscopy in Diagnosis of Dermal Disorders' during XXIX Annual Meeting of EMSI and National Conference on Electron Microscopy and Allied Fields held at University of Delhi, Delhi from 26-28 November 2007.

11. Delivered talk on 'A comparison of rapid and routine- processing of biological samples for EM'during XXIX Annual Meeting of EMSI and National Conference on Electron Microscopy and Allied Fields held at University of Delhi, Delhi from 26-28 November 2007.

12. 'A modified technique for localization of Alkaline Phosphatase under EM during XXIX Annual Meeting of EMSI and National Conference on Electron Microscopy and Allied Fields held at University of Delhi, Delhi from 26-28 November 2007.

13. 'Ultrastructural study of sperm in infertile males' during XXIX Annual Meeting of EMSI and National Conference on Electron Microscopy and Allied Fields held at University of Delhi, Delhi from 26-28 November 2007.

14. 'Ultrastructure of trophoblast in Hydatidiform' Moles during XXIX Annual Meeting of EMSI and National Conference on Electron Microscopy and Allied Fields held at University of Delhi, Delhi from 26-28 November 2007.

15. Examiner M.Sc. and Ph.D (Dairy Microbiology) of National Dairy Research Institute (Deemed University), Karnal.

16. Meetings of the Screening Committee for registration of Public Funded Research Institutions/Universities at DSIR, Technology Bhavan, New Delhi.

17. Dialogue on Environmental Health Crisis in Punjab organised by Environmental Health Action Group, Kheti Virasat Mission, Jaitu at Oswal Cancer Hospital, Ludhiana on January 5-6, 2008.

18. 'New Tools for Human Environmental Bio-monitoring' during Dialogue on Environmental Health Crisis in Punjab organised by Environmental Health Action Group, Kheti Virasat Mission, Jaitu at Oswal Cancer Hospital, Ludhiana on January 5-6, 2008.

19. Lecture on Promotion of Hindi entitled "Sangh Ki Rajbhasha Niti" by Dinesh Chandra Tripathi on Jan 11, 2008 at Institute of Pathology, New Delhi.

20. A lecture delivered by Nobel Laureate David Baltimore entitled "New Avenues to Health" on Jan 14, 2008 at Teen Murti Auditorium, Teen Murti House, New Delhi.

21. Technical Committee Meeting of National JALMA Institute for Leprosy and Other Mycobacterial Diseases, Agra on 7th Feb. 2008.

22. International Conference on Microbicides at Convention Hall, Hotel Ashok, New Delhi on Feb. 27, 2008.

23. Second International Symposium on "Recent Advances in Cardiovascular Sciences" at Delhi Institute of Pharmaceutical Science and Research, New Delhi on Feb. 28, 2008.

24. 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.

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

26. 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.

27. M.Sc. Dissertation supervised: 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.

28. 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.

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

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

31. 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.

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

33. Paper Presented '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.

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

35. 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.

36. 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.

37. 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.

38. 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.

39. 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.

40. 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.

41. 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.

42. Conducted WHO-sponsored Basic level and Advanced level training courses for technicians at Institute of Pathology from February 16 to March 14, 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 25th Sept. 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 30th July 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. Delivered a talk "Bioengineering of CEA from adult epidermal stem cells" as an Invited speaker and participated in a panel discussion on Stem Cells and Therapy at the one day Symposium on 'Therapeutic Potential Of Stem Cells' held to commemorate the IInd anniversary of Nichi-In Center for Regenerative Medicine on 27th of October 2007 at Chennai.

2. Delivered a talk entitled "Clinical applications of stem cells in burns" at "Indo-UK Seminar On Regenerative Medicine And Stem Cell Applications" as an Invited speaker and participated in a panel discussion on Regulatory & Ethical issues of Stem Cells and Applications of Stem Cells in the Pharma and Biotech Industry on 29th November 2007 at Stem Cell Unit, Life Line Hospitals, Chennai.

3. Teaching Faculty of Hands-On-Training Course of 12th ADNAT convention on "Basic Techniques in Stem Cell Biology: Isolation, Maintenance and differentiation" held at Center for Cell and Molecular Biology, Hyderabad between 25th February and 10th March 2008.

4. Participated in an interactive session to discuss the "New Developments In IPR Scenario In India" organized by Corporate Law Group at National Academy for Agricultural Sciences Complex, New Delhi on 29th April 2008.

5. 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" held at the Centre for Genetic Diseases and Molecular Biology, Department of Biochemistry, Pt. JNM Medical College, Raipur on 25th July 2008.

6. Invited to deliver a talk entitled "Clinical Potential of Unipotent Adult Epidermal Stem Cells" at Stem Cell Conference organized by Stem Cell Voice of India held at Medifest'08, Pragati Madan, New Delhi on 6th December 2008.

7. Invited to deliver a talk entitled "Burn Injury: A Burning Problem in Developing Countries and A Nightmare For Tissue Engineers" at a symposium of Biotechnology Research held as part of XXXII Indian Social Science Congress, between December 18-21, 2008, at Department of Biotechnology, Jamia Millia Islamia.

8. Teaching faculty and examiner for the off-campus pre-PhD programme of Birla Institute of Technology and Science, Pilani and undertook a course on "Cell and Tissue culture"- Course No.BIO G641.

Dr. Anju Bansal

1. Participated in National Symposium on Lung Pathology on 5th April 2007 at Vallabhbhai Patel Chest Institute , Delhi.

2. Paper presented at XXII Annual IAPM, 2007, Delhi Chapter on "Study of androgen receptor as a predictive marker in locally advanced breast cancer".

3. Paper presented at XXII Annual IAPM, 2007, Delhi Chapter on "Study of expression of mismatch repair genes in breast cancer in correlation with clinico-pathological parameters and response to neoadjuvant chemotherapy".

4. Presented poster at XXII Annual IAPM, 2007, Delhi Chapter on "Nodular melanoma, a distinct entity".

5. Presented poster at XXII Annual IAPM, 2007, Delhi Chapter on "Primary lymphoma of prostate".

6. Organized and conducted Symposium at the Institute on Cytology Session (Liquid based preparations - An update) and Histopathology Session (Current issues in diagnostic breast pathology) (by Dr. Syed Hoda, USA and Dr. Rana Hoda ,USA).

7. Participated in "Update in Surgical Pathology 2007" at Tata Memorial Hospital, Mumbai from 31st Aug. - 2nd Sep. 2007.

8. Organized and conducted Quarterly meeting of Delhi Chapter , IAPM on 8th Sept 2007 at Safdarjang hospital campus.

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. AP Singh

1. Presented poster at XXII Annual IAPM, April 2007, Delhi Chapter on "Neurothekeoma in the leg".

2. UICC-ICRETT Fellowship availed at TARP laboratory, National Cancer Institute, NIH, USA from 20th August 2007 to 20th September 2007.

Dr. VP Singh

1. Attended workshop on Microwave Sample Preparation Technique organized by Nulab equipment company at Central Revenue Control Laboratory, IARI, Pusa Campus New Delhi on 30th Jan. 2008.

2. Presented Poster at 'TraceSpec2007: 11th Workshop on Progress in Analytical Methodologies for Trace Metal Speciation jointly organized by the University of Munster Institute of Inorganic and Analytical Chemistry, the European Institute for Speciation Analysis and the International Association of Environmental Analytical Chemistry, Munster Germany during 4th –7th September 2007

3. Invited to deliver a talk at consultations workshop on "Entrepreneurship Possibility in Neutraceutical Food and Herbal Products: Production, Quality Assurance & Marketing" organized by Mahatma Gandhi Institute of Rural Industrialization (MGIRI), Wardha from 18th –22nd September 2007.

4. Invited to deliver a talk "Advance technique to estimate metallic contamination in food drug and environment" at ICMR sponsored National Workshop on Advance Techniques of Molecular Biology organized by the Department of Medicinal Chemistry, Institute of Medical Sciences, Banaras during 8th –10th Feb. 2008.

5. Post-Graduate Dissertation thesis supervised and successfully completed :

   Name of Student: Vinod Kumar

   Topic: Role of environmental toxicants especially heavy metals in spontaneous abortion

Dr. Raju Bagadi

1. Attended Bioinformatics Training course (funded by ICMR) conducted at Institute of Bioinformatics and Applied Biotechnology, Bangalore, during 3rd –25th August 2008.

Dr. AK Mishra

1. Attended '56th International Statistical Institute Conference' at Lisbon-Portugal from 22nd - 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 on 26th – 28th October, 2007 and presented paper entitled "Statistical Considerations in Breast carcinoma-A Study on Association of Androgen Receptors with Clinical Response".

7. 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.

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

Dr. L.C. Singh

1. Attended 21st Annual Conference of Indian Association of Pathologists and Microbiologists, Delhi Chapter on 16th April, 2006.

2. Attended seminar on Applied Biosystems Real time PCR technology on 8th May, 2006.

3. Attended Hitachi Spectroscopy Seminar on 12th September, 2006.

4. Attended a training programme on application of 3130XL Genetic Analyzer from 9th-11th October, 2006.

DNB Programme

The DNB programme at the Institute of Pathology continued with the admission of new students to the programme and successful completion of the training course by the final year students. During the year 2007-09, **six** students were admitted in the DNB course and **five** students have cleared the theory exam.

Year	Admission	Completion
2007-08	Dr. Binita Sinha Dr. Pooja Gupta Dr. Shweta Agarwal Dr. Harsh Hora	Dr. Kavita Jain Dr. Himanshu Vinod Rai
2008-09	Dr. Disha Arora Dr. Ila Jain	Dr. Punita Rao Dr. Monika Sharma Dr. Mallika Dixit

Half yearly appraisal as required by the NBE guidelines were held regularly during the period under report and the following local experts were present at the Institute for the same :

Year	Expert
July 2007	Dr. Tejinder Singh
Feb. 2008	Dr. Madhu Tatke
July 2008	Dr. Amit Dinda

Ph.D Programme

The Institute supports an active Ph.D program with recognition from BITS-Pilani, Jiwaji University, Gwalior and IP University, Delhi. During the period 2007-09, following three students were awarded Ph.D and two students submitted thesis.

1. **Mr. Subba Raju** was conferred with the degree of Doctorate of Philosphy by Jiwaji University, Gwalior.

2. **Mr. Nasim Akhtar Ansari** was conferred with the degree of Doctorate of Philosphy by Jiwaji University, Gwalior.

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

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

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

Institute of Pathology further attracted young Junior/Senior Research Fellows obtaining fellowships through various agencies for Ph.D.

	2007-08	2008-09
CSIR	1	6
ICMR	10	6
UGC		6

Training Programme

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

Under the Training Programme, total **Twelve** students deputed by **Jiwaji University, Gwalior** underwent research training for doing six-months project work in different laboratories at Institute of Pathology: Subsequent to completion of their project work, the students will submit their dissertations.

1. Ms. Swarna Saxena
2. Mr. Ragini Chaturvedi
3. Ms. Akansha Singh
4. Ms. Shardhanjali Srivastava
5. Ms. Pooja Narang
6. Ms. Bhupender Singh
7. Ms. Thangyam Jeena Devi
8. Ms. Ankur Saxena
9. Mr. Ratnam Prasad
10. Mr. Urja Jaiswal
11. Ms. Sonia
12. Ms. Priyanka

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 ADVISORY COMMITTEE

SCIENTIFIC ADVISORY COMMITTEE

1	**Dr. Indira Nath**, Emeritus Professor, Raja Ramanna Fellow, 707, S P. Apartments, Sarvpriya Vihar, New Delhi.	Chairperson
2	**Dr. Rajiv Sarin, Director**, Tata Memorial Centre, Advance Center for Treatment, Research & Education in Cancer, Kharghar, Navi Mumbai – 410 210.	Member
3	**Dr. Kusum Joshi, Professor & Head**, Department of Pathology, Post Graduate Institute of Medical Sciences, Chandigarh.	Member
4	**Dr. Chitra Sarkar, Professor**, Department of Pathology, All India Institute of Medical Sciences, Ansari Nagar, New Delhi -29	Member
5	**Dr. Subroto Sinha, Professor**, Department of Biochemistry, All India Institute of Medical Sciences, Ansari Nagar, New Delhi – 110 029.	Member
6	**Dr. N.K. Mehra, Professor**, Department of Biophysics, All India Institute of Medical Sciences, Ansari Nagar, New Delhi – 110 029.	Member
7	**Dr. R.R. Bhonde, Scientist 'F'** National Centre for Cell Science, NCCS Complex, University of Pune Campus, Ganeshkhind, Pune – 411 007.	Member
8	**Dr. Ravi Sirdeshmukh**, Director-Grade Scientist (Sci G) Head, Proteomics Lab, Center for Cellular & Molecular Biology, (Council of Scientific & Industrial Research), Uppal Road, Hyderabad – 500 007.	Member

9	**Dr. Sudha Bhatacharya,** Department of Environmental Study, Jawahar Lal Nehru University, New Delhi.	Member
10	**Dr. Dhananjaya Saranath, Director Research** Reliance Life Sciences (Pvt.) Ltd., Dhirubhai Ambani Life Sciences Centre, R-282, TTC Area of MIDC, Tale Hane Lanapure Road, Navi Mumbai - 400 701.	Member
11	**Dr. Kiran Katoch, Director-in-Charge** National Jalma Institute of Leprosy and other Microbacterial Diseases, Taj Ganj, Agra, UP	Member
12	**Dr. Chandrima Saha, Staff Scientist – VII,** National Institute of Immunology, Aruna Assaf Ali Marg, New Delhi.	Member
13	**Dr. Shyamal Roy**, Indian Institute of Chemical Biology, Council of Scientific & Industrial Research, 4, Raja SC Mullick Road, Kolkata – 700 032, West Bengal.	Member
14	**Dr. Ashwini Kumar**, Industrial Toxicology Research Centre, Post Box No. 80, Mahatma Gandhi Marg, Lucknow – 226 001.	Member
15	**The Principal & Medical Superintendent,** Vardhman Mahavir Medical College & Safdarjang Hospital, New Delhi – 110 029.	Member

ANIMAL ETHICAL COMMITTEE

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IOP Members

Dr. Aruna Singh
Dr. Sangita Rastogi
Dr. Poonam Salotra
Dr. L. K. Yerneni
Dr. Usha Agrawal

External Members

Scientist Member

Dr. J. K. Batra
Sr. Scientist,
National Institute of Immunology,
New Delhi

Social Scientist Member

Prof. M. N. Panini
Professor,
School of Social Sciences,
Jawaharlal Nehru University,
New Delhi

Veterinary Consultant

Dr. P.K. Yadav
Sr. Veterinary Officer,
Experimental Animal Facility,
AIIMS, New Delhi

CPCSEA Nominee

Dr. Ravindra Sharma
C-7, MCD Officers Flats,
'R' Block, Gr. Kailash-I,
New Delhi

INSTITUTIONAL COMMITTEES

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Dr. A. K. Jain
Dr. L K. Yerneni
Mrs. R. Saratha
Mr. V.K. Khanduja

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Dr. Sangita Rastogi
Dr. Purnima Paliwal
Dr. BS Raju
Ms. R. Saratha
Mr. Raja Ram

Library Committee

Dr. Sujala Kapur
Dr. Saurabh Varma
Dr. Usha Agrawal
Dr. Avninder P Singh
Mrs. R Saratha
Ms. Anita Sharma

Maintenance/Security and Sanitation Committee

Dr. Poonam Salotra
Dr. Ranvir Singh
Dr. AP Singh
Dr. Ashwani Kr. Mishra
Mrs. R. Saratha

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Dr. Saurabh Varma
Dr. BS Raju
Mrs. R. Saratha
Mr. V.K. Khanduja

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Dr. Saurabh Varma
Dr. Ranvir Singh
Mrs. R Saratha
Mr. VK Khanduja

Renovation/Building Committee

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Mr M.M. Prasad
Mr. AK Srivastava
Dr. Aruna Singh
Dr. S. Kapur
Dr. A.K. Jain
Dr. Usha Agrawal
Dr. Ashwani Kr. Mishra
Mrs. R. Saratha
Mr. V.K. Khanduja

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Mr M.M. Prasad
Mr. AK Srivastava
Dr. Sangita Rastogi
Dr. Poonam Salotra
Dr. LK Yerneni

Technical Committee

Dr. Sayed Akhtar Hussain
Dr. SN Das
Dr. Chandrima Saha
Dr. Aruna Mittal
Dr. A.K. Jain
Dr. L.K. Yerneni
Mrs. R. Saratha
Mr. V.K. Khanduja

Fire Fighting Committee

Dr. Ranvir Singh
Mr. AK Bagga
Mr. Raja Ram

Canteen Committee

Dr. Sangita Rastogi
Dr. Anju Bansal
Dr. Ashwani Kr. Mishra
Mrs. R Saratha
Mr. Ravi Kapoor
Ms. Madhu Badhwar

STAFF LIST

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SCIENTIFIC STAFF

1.	Dr. Sunita Saxena, MBBS,DCP, MD (Path)	Director
2.	Dr. Aruna Singh, M.Sc., Ph.D.	Scientist 'F'
3.	Dr. Sujala Kapur, MBBS, MD (Path & Microbiology)	Scientist 'E'
4.	Dr. Sangita Rastogi, M.Sc., M.Phil., Ph.D. (Zoology)	Scientist 'E'
5.	Dr. Poonam Salotra, M.Sc.(Biochem), Ph.D. (Biochem)	Scientist 'E'
6.	Dr. A. K. Jain, M.Sc.(Dairy Bacteriology), Ph.D.	Scientist 'E'
7.	Dr. Ranvir Singh, MBBS	Scientist 'D'
8.	Dr. A. K. Bagga, MBBS	Scientist 'D'
9.	Dr. L. K. Yerneni, M.Sc., Ph.D.	Scientist 'D'
10.	Dr. Usha Agrawal, MBBS, MD (Path)	Scientist 'D'
11.	Dr. Anju Bansal, MBBS, MD (Path)	Scientist 'C'
12.	Dr. Saurabh Verma, M.Sc., Ph.D.	Scientist 'C'
13.	Dr. Purnima Malhotra, MBBS, MD (Path)	Scientist 'C'
14.	Dr. Avninder Pal Singh, MBBS, MD (Path)	Scientist 'C'
15.	Dr. S. Appala Raju Bagadi, M.Sc., Ph.D.	Scientist 'B'
16.	Dr. A. K. Misra, Ph.D. (Statistics)	Scientist 'B'

TECHNICAL STAFF

1. Mrs. Asha Rani Srivastava, Technical Officer
2. Dr. L.C. Singh, Technical Officer
3. Mr. Moti Lal, Technical Officer
4. Mrs. Madhu Badhwar, Technical Officer
5. Mr. Shiv Prakash, Data Processing Assistant (Gr. 'B')
6. Mrs. Seema Sharma, Data Processing Assistant (Gr. 'A')

7. Mr. Pushp Raj, Dark Room Assistant
8. Mrs. Asha Rani, Technical Assistant
9. Mr. Chandi Prasad, Technical Assistant
10. Mrs. Anita Bhatia, Technical Assistant
11. Mrs. Karuna, Technical Assistant
12. Mrs. Krishna, Technical Assistant
13. Mr. Kuldeep Kumar Sharma, Lab. Technician
14. Mrs. Valsamma Mathew, Lab. Technician
15. Mr. Ishwar Singh, Lab. Technician
16. Mr. Jagdish Pant, Lab. Technician
17. Mr. P.D. Sharma, Lab. Technician
18. Mr. Rameshwar, Lab. Assistant
19. Mr. Suresh Bhimrao Kamble, Lab. Technician
20. Mr. Satyapal Singh, Lab. Technician
21. Mr. Madan Lal, Lab. Assistant
22. Mr. Shiv Bahadur, Lab. Assistant
23. Mr. Dharampal, Driver-cum Mechanic
24. Mr. Sushil Kumar, Driver
25. Mr. Manwar Singh, Driver
26. Mr. Puran Singh, Messenger-cum-Driver
27. Mr. Shyam Sunder, Lab. Attendant
28. Mr. Ajit Singh Lehra, Lab. Attendant
29. Mr. Daya Sagar, Lab. Attendant
30. Mr. Bala Dutt, Lab. Attendant
31. Mr. Jagdish Ram, Lab. Attendant

ADMINISTRATIVE STAFF

1. Mrs. R.Saratha, Administrative Officer
2. Mr. V.K. Khanduja, Accounts Officer
3. Mr. Raja Ram, S.O.
4. Mr. Ravi C. Kapoor, S.O.
5. Mr. Yogendra Kumar, S.O.
6. Mrs. Ganga Misra, P.S.
7. Mr. Jagdish Prashad, P.A.
8. Ms. Rekha Rani, P.A.
9. Mrs. Anita Sharma, Assistant (Librarian)
10. Mrs. Sunita Ahuja, Assistant
11. Mr. V. S. Rawat, Assistant
12. Mr. Dashrath G. Khambadkar, Assistant
13. Mr. Subhash Babu, Assistant
14. Mr. Mangey Ram, Assistant
15. Mrs. E. Sharda, Stenographer
16. Mrs. Sangeeta Batra, Jr. Librarian
17. Mrs. Sushma Ralhan, U.D.C.
18. Mrs. Sharmila Kamra, U.D.C.
19. Mr. Ajay Joshi, U.D.C.
20. Mrs. Sonia Khattar, U.D.C.
21. Mr. Rajesh Kumar, U.D.C.
22. Mrs. Santosh Deora, Receptionist-Cum-Tele.Operator
23. Mr. Brijender Singh, LDC
24. Mrs. Archana, LDC
25. Mr. Rajender Kumar, Sr. Gestetner Operator
26. Mr. Anil Kumar Verma, Gestetner Operator
27. Mr. Jagdish, Lab. Assistant

28. Sh.Bijendra Kumar, Lab.Assistant
29. Ms. Jyoti, LDC
30. Sh.Kamal Dev, Lab.Assistant
31. Mr. Dharmendar Singh, Attender
32. Mr. Ram Chander Das, Attender
33. Mr. Puran Chand, Attender
34. Mr. Rajendra Kumar, Attender
35. Mr. Manoj Kumar, Attender
36. Mr. Anish Kumar Saxena, Attender
37. Mr. Raj Singh, Attender
38. Mr. Sajid Hussain, Lab. Attendant
39. Mr. Chandrika Prasad, Attender
40. Mr. Sanjay Dutt Upreti, Attender
41. Mrs.Charanjit Kaur, Attender

INSTITUTE OF PATHOLOGY, NEW DELHI