ANNUAL REPORT

2011-12

NATIONAL INSTITUTE OF PATHOLOGY (ICMR)
P.O. Box 4909, Safdarjang hospital campus, New Delhi-110 029
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NATIONAL INSTITUTE OF PATHOLOGY (ICMR)

Executive Summary

During the year under report, while Indian Council of Medical Research was celebrating its 100 years of service to the nation, the Institute of Pathology achieved another milestone by attaining the status of National Institute of Pathology on 1st May 2012, its Foundation Day, based on research achievements and services provided to the nation. Scientists made seminal contributions by developing simple non-invasive, sensitive and indigenous diagnostic assays as well as identified vaccine candidates for *Leishmania* and *Chlamydia*, established technologies for epidermal expansion for auto-transplantation in burns patients, breast cancer cell lines from primary tumor of young women and *in vitro* model to assess cytotoxicity to tumor cells in bladder cancer to give personalized therapy. Microarray and functional genomics studies have also been carried in leishmaniasis to understand mechanism of drug resistance and in tobacco-associated cancers in the north-east to unravel the molecular pathogenesis and to identify new biomarkers for risk and progression. Besides, various outreach programs have also been initiated during the year under report by conducting workshops for training of medical doctors at various medical colleges for research sensitization.

The focus of research in Tumor Biology Division had been on identification of diagnostic, predictive and prognostic biomarkers, new drug targets and gene-environmental links mainly in **Breast, Prostate, Urinary Bladder and Brain cancers, Lymphomas and Leukemia and cancers in North-east region of India.** Investigation of molecular pathogenesis of early onset breast cancer by gene expression and methylation profiles has led us to the identification of several gene families, *viz.:* SLC, TMEM, cadherins, FOX, chromatin modification and replication that are differentially expressed in early onset or late onset cancers. Functional studies to understand their role are underway in two breast cancer cell lines that have been established by us from primary breast tumors in young women. Study of breast cancer stem cells in various breast cancer / normal cell lines is undergoing for investigating their role in drug resistance and

metastasis. Study is also undergoing to asses AR-mediated transcription and cell growth in both AR (+) and AR (-) breast cancer cells through the knock-down of AR expression and the effect of anti-cancer drugs on AR signaling pathways in association to drug response.

Gene expression studies to characterize the host immune profile associated with progression of superficial TCC of urinary bladder revealed upregulation of IL-17A, Th2 cytokines- IL-4 and IL-9, INH and Stat1 genes and downregulation of SOCS1 and IL-6R. In Non-Muscle Invasive Urothelial Cancer (*NMIUC),* the expression profile showed Th2 dominance while in Muscle Invasive Urothelial Cancer *(MIUC),* the Th1/Th2 balance showed polarization towards Th1. Gene expression of NFκB pathway showed downregulation of EDARADD and HTR2B in both *NMIUC* and *MIUC*. Akt1 was found downregulated in *MIUC* and TBK1 was found upregulated in *NMIUC* indicating the activation of the non-canonical NFκB pathway. A tissue micro-array based screening of 195 gliomas for IDH1 expression has shown IDH1 to be a useful immunohistochemical marker to differentiate reactive gliosis from low-grade astrocytoma and primary from secondary glioblastoma. Studies have been initiated to characterize the epigenetic modifications associated with Acute Myeloid Leukaemias and if there is any correlation between epigenetic modifications and cytogenetic abnormalities. Studies have also been undertaken to study the effect of ROS on B-cell activation and proliferation in Chronic Lymphocytic Leukaemias.

Studies have been continued to further investigate the role of tobacco and pesticide for high incidence of Oral, Lung and Gastric cancers in North-east region. Targeted re-sequencing of 169 functionally relevant and potentially important genes in oral cancer showed large number of mutational changes including 96 SNPs (50 novel and 46 known SNPs) and 46 InDels (29 novel and 17 known InDels). Most of the known SNPs with high read depths (>50) were located in tumor suppressor genes in addition to some SNPs in non-coding regions. Application of multi-analytical approach, including Logistic Regression (LR), Classification And Regression Tree (CART) and Multifactor Dimensionality Reduction (MDR) to explore high order interactions among xenobiotic metabolizing genes and environmental risk factors in lung cancer cases showed smoking as the predominant risk factor and identified two distinct predictor models for the risk of lung cancer in smokers (tobacco chewing, EPHX1 Tyr113His and SULT1A1 Arg213His) and non-smokers

(CYP1A1*2A, GSTP1 Ile105Val and SULT1A1 Arg213His) with Testing Balance Accuracy (TBA) of 0.6436 and 0.6677, respectively. In gastric cancers, targeted resequencing showed total of 178 SNPs and 23 indels (12 insertions and 11 deletions). Most of the observed SNPs were known SNPs, however, a few novel SNPs were also found in the in-coding regions of genes that may have functional relevance in the pathogenesis of gastric cancer. Study on immunogenetic profile of Nasopharyngeal Carcinoma in North-east region by preliminary analysis of microsatellite markers by using Gene Mapper software showed many allelic variants for the all the thirty-one microsatellite markers studied. For detection of EBV in tissue / blood samples optimization of PCR conditions has been done.

At NIP, efforts have been continued to develop non-invasive, simpler and rapid diagnostic methods for both visceral leishmaniasis and Post Kala-azar Dermal Leishmaniasis (PKDL) and during the year under report, slit aspirate sample were introduced for both immunological and molecular-based PKDL diagnosis. Q-PCR determined parasite load in slit aspirate and tissue biopsy was found to be a minimum-invasive, simple, rapid and reliable assay for diagnosis of PKDL, subsequent to initial screening with slit aspirate rk39 strip test, with potential for field application and assessment of cure. In a study conducted at NIP, oral miltefosine (50 mg thrice daily for 60 days or twice daily for 90 days) has been shown to have a beneficial effect in PKDL in a larger series of patients with initial cure rate of 96% (95% confidence interval, 79–99%). Sixty one percent patients were cured with 50 mg thrice daily, 50% in 60 days and 11% within 90 days. Treatment was safe with no relapses at 1-year follow-up. Thus, oral miltefosine, 50 mg thrice daily for 60 days or twice daily for 90 days, could be an effective treatment for PKDL. Studies on drug susceptibility in *Leishmania* isolates following Miltefosine treatment in cases of Visceral Leishmaniasis and PKDL using an amastigote-macrophage model showed significantly higher tolerance ($p<0.0001$) to Miltefosine in PKDL than in VL isolates. Point mutations in the Miltefosine transporter (LdMT) and its beta sub-unit LdRos3 genes previously reported in parasites with experimentally induced MIL resistance were not present in the clinical isolates. Further, the mRNA expression profiles of these genes were comparable in the pre- and post-treatment isolates of VL and PKDL.

To understand the precise immunological cause of PKDL, investigation of ex-vivo mRNA and protein analysis of natural regulatory T-cells (nTreg) markers (Foxp3, CD25 and

CTLA-4) and IL-10 levels in lesional tissues of PKDL patients at pre- and post-treatment stages showed significantly elevated mRNA levels of nTreg markers and IL-10 in pre-treatment PKDL cases compared to controls which were restored after treatment. The accumulation of nTreg cells was evident as Foxp3 positive cells in lesional tissues of PKDL patients. Analysis of nTreg cell markers and IL-10 in different clinical manifestations of disease revealed elevated levels in nodular lesions compared to macules / papules. Further, Foxp3, CD25 and IL-10 mRNA levels directly correlated with parasite load in lesions / tissues. Data demonstrated accumulation of nTreg cells in infected tissue and a correlation of both IL-10 and nTreg levels with parasite burden suggesting their role in disease severity in PKDL.

In genital chlamydiasis, study on role of cox-2 in recurrent aborters showed upregulated (2.0-fold) mRNA expression of cox-2 gene in *C. trachomatis* infected women undergoing recurrent spontaneous abortion in comparison with women undergoing MTP and found *C. trachomatis*-negative. The study is continuing to quantitate the chlamydial load by real-time PCR in spontaneous aborters with increased cox-2 expression and to compare the prostaglandin E receptor genes expression pattern in infected recurrent spontaneous aborters and uninfected women undergoing surgical abortion. Study on the role *C. trachomatis* in immunopathogenesis of reactive arthritis / axial spondyloarthritis showed 21.05% ReA / AS patients positive for *C. trachomatis* MOMP / plasmid genes by conventional PCR. *C. trachomatis*-specific IgA antibodies were found in the synovial fluid of 31.5% patients while 15.7% patients were positive for serum anti-*C. trachomatis* IgA antibodies.

After effectively demonstrating 'numerical' dose-dependant approach of MMC co-culture model comprising of NIH 3T3 fibroblasts as feeders and human epidermal keratinocytes as target stem cells, a similar approach was initiated by using Swiss 3T3 cells as feeders with the aim to standardize the complex protocol of generating human Cultured Epidermal Autograft (CEA) towards clinical application in burns patients. Concomitant to the arithmetically calculated dose dependant cellular extinction of Swiss 3T3 cells, the human epidermal keratinocytes plated along with these feeders showed similar dose dependent cell proliferation but in a negatively correlated fashion. Additionally, the basic methodology of preparing the organotypic co-culture skin model consisting of 3T3 fibroblasts in Type-I collagen gel as DE and an epithelialization of the same

using human epidermal keratinocytes has been accomplished. The differentially growth arrested 3T3 cells have exhibited periodic cell extinction patterns similar to what was observed with 2-D culture technique.

Investigation on the role of phthalate in male infertility showed presence of two metabolites, *i.e.* MEHP and MBP in urine samples with average level of MEHP to be 113.46 µg/ml and that of MBP as 138.19 µg/ml in infertile subjects suggesting their potential role as biomarker for exposure. Study on the effects of pesticides exposure on the health of farm-workers in tea-gardens in North-east states showed presence of organophosphorus pesticides such as acephate, parathion, malathion, ethion and phosalone, in addition to organo-chlorine pesticides, *viz.:* heptachlor, DDT, chlordane in the placental tissue as well as in the maternal and cord blood samples with correlation to different clinical manifestations such as headaches, dizziness, blurred vision, nausea and vomiting, stomach cramps, diarrhoea, excessive salivation and sweating, tightness of the chest, muscle twitching, etc. Estimation of Cd & Pb in the placental digest, maternal & cord serum digest from intra-uterine growth restricted deliveries showed significantly high concentration of Cd & Pb in all the three biological samples suggesting inability of placenta to act as a perfect barrier for toxic elements.

Subsequent to becoming a national institute, the scientists have taken up additional responsibility to initiate outreach programs for educating young medical faculty and post-graduate students in various medical colleges in research methodologies, which is expected to boost research in medical colleges. This has met with a good response. Besides this, we have continued our human resource development activities by training Ph.D. and DNB candidates. I take this opportunity to convey my sincere thanks to Dr. V.M. Katoch for his support and encouragement for scientific and academic programs at NIP and also acknowledge the team work of my scientific, technical and ministerial staff for attaining this status.

Dr. Sunita Saxena

Director

LIST OF ONGOING PROJECTS

TUMOR BIOLOGY

BREAST CANCER

1. STUDY ON GENE EXPRESSION AND HYPERMETHYLATION PROFILES IN EARLY ONSET BREAST CANCER
2. STUDY ON THE EFFECTS OF ANTI-CANCER DRUGS ON BREAST CANCER STEM CELLS
3. STUDY ON THE EFFECT OF siRNA MEDIATED ANDROGEN RECEPTOR GENE SILENCING ON ANDROGEN SIGNALING PATHWAY IN BREAST CARCINOMA

URINARY BLADDER CANCER

1. CHARACTERISATION OF HOST IMMUNE PROFILE ASSOCIATED WITH PROGRESSION OF SUPERFICIAL TCC OF URINARY BLADDER BY MICROARRAY ANALYSIS
2. TO STUDY THE ROLE OF CYCLOOXYGENASES IN CYTOKINES DYSFUNCTION OF INVASIVE AND NON-INVASIVE TCC OF HUMAN BLADDER

CANCERS IN NORTH-EAST INDIA

1. EFFECT OF TOBACCO AND PESTICIDE IN CANCERS IN NORTH-EAST INDIA
2. GENOME-WIDE ANALYSIS OF GENETIC ALTERATIONS IN PATIENTS WITH OESOPHAGEAL CANCER FROM NORTH-EAST INDIA USING SINGLE NUCLEOTIDE POLYMORPHISM ARRAYS
3. IMMUNOGENETIC PROFILE OF NASOPHARYNGEAL CANCER IN A HIGH-PREVALENCE REGION OF NORTH-EAST INDIA

HEMATOPOIETIC-LYMPHOID MALIGNANCIES

1. GENETIC AND EPIGENETIC PROFILE OF ACUTE MYELOID LEUKEMIA
2. REDOX REGULATION OF LYMPHOCYTE SIGNALING IN B-CLL

BRAIN TUMORS

1. IMMUNOHISTOCHEMICAL EXPRESSION OF IDH1 IN GLIOMAS: A TISSUE MICROARRAY BASED APPROACH

PATHOLOGY OF INFECTIOUS DISEASES

LEISHMANIASIS

1. DRUG RESISTANCE IN VISCERAL LEISHMANIASIS
2. STUDIES ON IMMUNOLOGICAL RESPONSES TO RECOMBINANT PSA (PARASITE SURFACE ANTIGEN)- A POTENTIAL VACCINE CANDIDATE
3. IMMUNE RESPONSES AGAINST CENTRIN KNOCK OUT LIVE ATTENUATED LEISHMANIA PARASITES IN THE HUMAN CELLS
4. DIAGNOSIS OF VL AND PKDL USING LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP)

CHLAMYDIASIS

1. ROLE OF OXIDATIVE STRESS IN CHLAMYDIA TRACHOMATIS INFECTED FIRST TRIMESTER SPONTANEOUS ABORTERS
2. IMMUNOMOLECULAR EXPRESSION OF CYCLOOXYGENASES AND PROSTAGLANDIN RECEPTORS IN ENDOMETRIAL CURETTAGE TISSUE OF CHLAMYDIA TRACHOMATIS INFECTED WOMEN DURING SPONTANEOUS ABORTION
3. IMMUNOPATHOGENESIS OF REACTIVE ARTHRITIS INDUCED BY CHLAMYDIA TRACHOMATIS

LEPROSY

1. CLINICOPATHOLOGICAL DETERMINANTS IN LEPROSY TYPE 1 REACTIONS

TUBERCULOSIS

1. UNDERSTANDING THE UNDERLYING MECHANISM OF MACROPHAGE IMMUNE MODULATION AND IDENTIFICATION OF MARKERS FOR TB TREATMENT END POINT DETERMINATION

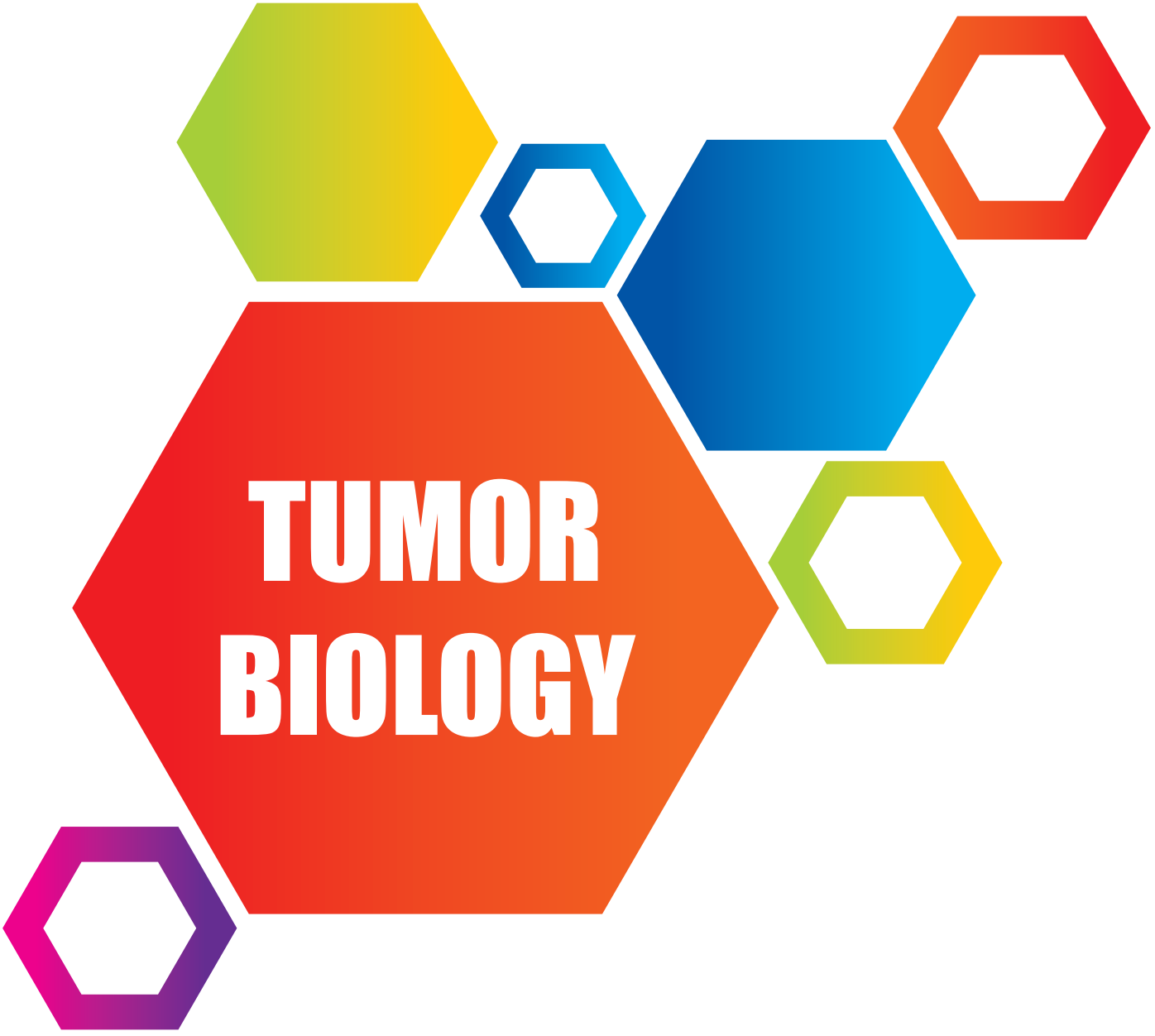
ADULT STEM CELL BIOLOGY

1. A NOVEL ARITHMETIC APPROACH FOR FOOL-PROOF PRODUCTION OF GROWTH ARREST IN 3T3 CELLS SUITABLE FOR HUMAN EPIDERMAL CULTURE
2. STUDIES ON EPITHELIAL-MESENCHYMAL INTERACTIONS USING HUMAN EPIDERMAL KERATINOCYTE STEM CELLS AND INNOVATIVELY GROWTH-ARRESTED FIBROBLAST FEEDERS IN 3-D COLLAGEN MODEL

ENVIRONMENTAL TOXICOLOGY

1. ASSESSMENT OF PESTICIDE EXPOSURE IN TEA GARDEN WORKERS OF NORTH-EASTERN STATE OF INDIA (HEBM)
2. DYNAMICS OF ULTRA-STRUCTURAL AND IMMUNOLOGICAL EVENTS IN RESPONSE TO TREATMENT IN DIFFERENT FORMS OF PSORIASIS: CO-MORBIDITIES ASSOCIATED WITH PSORIASIS


RESEARCH
ACTIVITIES


TUMOR
BIOLOGY

TUMOR BIOLOGY

BREAST CANCER

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

Scientific staff	:	Dr. Sunita Saxena, Dr. SA Raju Bagadi, Dr. Sujala Kapur, Dr. Anurupa Chakroborty, Ms Shreshtha Malvia
In collaboration with	:	Dr. D Bhatnagar, Consultant, Dr. Chintamani, Dr. RS Mohil, Dr. A Bhatnagar, Department of Surgery, Safdarjang hospital, New Delhi
Duration	:	2010-12

Aims, Objectives & Background

In India, an average of 100,000 women is diagnosed with carcinoma of the breast and 40,000 women die of the disease every year. Interestingly, although overall incidence of breast cancer in Indian population is low compared to western populations, the incidence of early onset disease (< 40 years) does not show significant geographic variation (ASR range worldwide of 12–33) (Parkin 1997) suggesting that in the Indian population, a greater proportion of breast cancer is due to early onset disease and occurs a decade earlier than in western populations (NCRP 2001a, Parkin 2002, NCRP 2001b). The average age of Indian breast cancer patients ranges between 44.2 - 49.4 years according to Cancer Registries at six hospitals and between 50-53 years according to population-based Registries, compared to 61-64 in US white females. Breast cancer diagnosed at young age is well recognized as clinically different from breast cancers diagnosed at older ages. Younger patients more frequently exhibit aggressive features such as large tumor size, high histologic grade, positive lymph nodes, absence of steroid receptors and high S-phase fraction, and young age itself has been shown to be an independent predictor of adverse prognosis. Although majority of Indian women are married and have children at young age and breast-feed their children, yet, the development of breast cancer at young age is an enigma which needs to be elucidated.

To elucidate genetic and epigenetic factors associated with early onset breast cancer in Indian women, study of gene expression profile and methylation profile was undertaken to identify unique molecular signature of breast cancer that occurs at younger age (< 40 years) with the goal to identify new biomarkers for breast cancer, particularly in young patients.

Work done during the year

In order to identify unique gene expression profiles associated with breast cancer that occurs at early age (<40 years) and late age (>55 years), we have analysed gene expression and methylation profiling of 20 early and 20 late onset breast tumors and 5 distant normal tissues were used as controls. The validation of gene expression data was done by using real time quantitative PCR. Genes involved in several pathways were differentially expressed in breast tumors, of these Wnt signalling pathway, chromatin modifying enzymes, cell adhesion molecules, CYP gene family members were significantly deregulated. The differentially expressed genes validated included c-MYC, EZH2, SMARCA3, CDH23, CYP46A1, CYP4F12, HMGB2 and SOX7 genes. Expression of these genes were validated in 20 breast tumors (10 early and 10 late onset) and compared with commercial normal breast total RNA and RNA from distant normal tissues. Among these genes, CDH23, CYP4F12 were downregulated in all tumors. JMJD4, EZH2, and c-MYC genes were upregulated in all tumors whereas SMARCA3, CYP46A1 were upregulated in late onset tumors (Figs. 1a-b). Expression of corresponding proteins in tissues is being analyzed currently in order to further validate the markers and determine their clinical significance of expression of these markers.

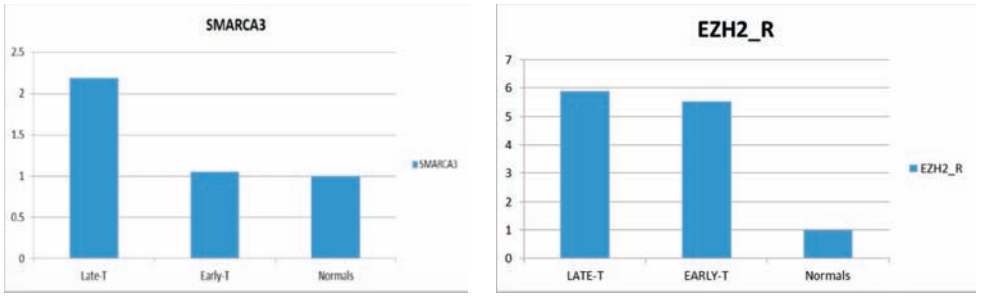


Fig. 1 a. Expression of SMARC3 in late and early onset tumors in comparison with RNA from normal mammary tissue. **b.** Expression of EZH2 in late and early onset tumors in comparision with RNA from normal mammary tissue.

2. STUDY ON THE EFFECTS OF ANTI-CANCER DRUGS ON BREAST CANCER STEM CELLS

Scientific Staff	**:** Dr. Sunita Saxena, Ms Shantilatha P.
Duration	**:** 2011-13

Aims, Objectives & Background

Tumor recurrence and treatment failure are well known in cancer therapy and recently, it has been linked with cancer stem cells (CSCs). The CSCs have the property of self renewal, differentiation in different progeny, slow growth and may not respond to standard treatment as bulk cancer cells. Breast Cancer Stem Cells (BCSCs) are increasingly thought to play a major role in breast cancer growth, relapse and metastasis. BCSCs, although comprise a small minority of tumour cells are responsible for drug resistance, hence it is important to assess the effect of therapeutic agents on bulk cancer cells as well as BCSCs. BCSCs are characterized by combined expression of cell surface markers $CD44^{+}/Cd24^{-}$. It has been recently reported that BCSCs can also be identified and sorted out based on the presence of Aldehyde dehydrogenase (ALDH1) enzyme.

Work done during the year

MCF7 breast cancer cell line, was grown as adherent culture. The cells were harvested when they are in their log phase of growth and were stained with aldefluor reagent using aldefluor kit as per the manufacturer's instructions.

Aldefluor assay and flow cytometry

Aldefluor assay was performed as per the manufacturer's instructions (*Stem Cell Technologies, Vancouver, BC, Canada*). Single cells obtained from cell cultures were incubated in Aldefluor assay buffer containing an ALDH substrate, bodipy-aminoacetaldehyde for 45 minutes at 37°C. As a negative control, a fraction of cells from each sample were incubated under identical condition in the presence of ALDH inhibitor, diethylaminobenzaldehyde (DEAB). Flow cytometry was conducted using FACS

ARIA II SORP (Special order research product) (*Becton Dickinson*). ALDEFLUOR fluorescence was excited at 488 nm and fluorescence emission was detected using a standard FITC 530/30 band pass filter. The sorting gates were established using the negative control.

Quantitation of Breast Cancer Stem Cells (BCSCs): A cell population with a high Aldehyde dehydrogenase (ALDH) enzyme activity was previously reported to enrich mammary stem / progenitor cells. Hence, Breast Cancer Stem Cells (BCSCs) were quantitated and sorted out based on the presence of the aldehyde dehydrogenase (ALDH) marker using Aldefluor assay. The $ALDH^{+/bright}$ cells were then sorted out using FACS ARIA II. Analysis by flow cytometry revealed that MCF7, breast cancer cell line have 0.2% of ALDH-positive BCSCs.

Mammosphere formation assay: ALDH-positive cells were seeded in low-attachment plates in media devoid of serum. Floating spheroids, termed mammospheres were observed within 6- 8 days of plating. Mammospheres were grown in serum-free medium supplemented with bFGF (*Gibco*), B27 (*Gibco*) and human recombinant insulin (*Sigma*) under low adherence conditions, as described by Dontu *et al*. The sorted out $ALDH^{+/bright}$ cells (termed as mammosphere Forming Units– MFUs) and the ALDH-negative cells were plated in six-well ultra low attachment plates (*Corning, MA*) in triplicates for the formation of primary mammospheres at a density of 2.5 x 10^3 cells/well. The resulting primary mammospheres after 10 days (termed M1 mammospheres) were collected by centrifugation at 500 rpm for 03 minutes. Mammospheres were counted and were dissociated both enzymatically and mechanically to obtain single cell suspension. The dissociated cells were sorted again using FACS. To test the functional definition of stem cells to self-renew, these MFUs enriched for breast stem and early progenitor cells were again plated after live cell count for mammosphere formation and were subjected to serial passaging every 10^{th} day leading to the generation of M2, M3, M4 mammospheres and so on. The mammospheres were counted after every passage and the images were recorded using ImagePro software. Since mammosphere formation was not observed in the ALDH-negative fraction, these cells were not plated in the subsequent passages (Fig. 1).

A dynamic increase in the number of MFUs / ALDH1 positive cells with increasing passages has been observed until the second generation (M2) followed by a gradual reduction by the seventh (M7) generation (Fig. 2). Similarly percentage of mammospheres

also increased till M2 passage and then declined by M7 passage after which mostly single cells and few clumps / aggregates of cells were seen.

Future work to be done

To estimate the proportion of BCSCs present in various breast cancer cell lines and to study the effects of anticancer drugs on the proliferation, differentiation and invasive capacities of CSCs in comparison with bulk cancer cells in order to correlate the association of ALDH-positive breast cancer stem cells with drug resistance and tumor relapse.

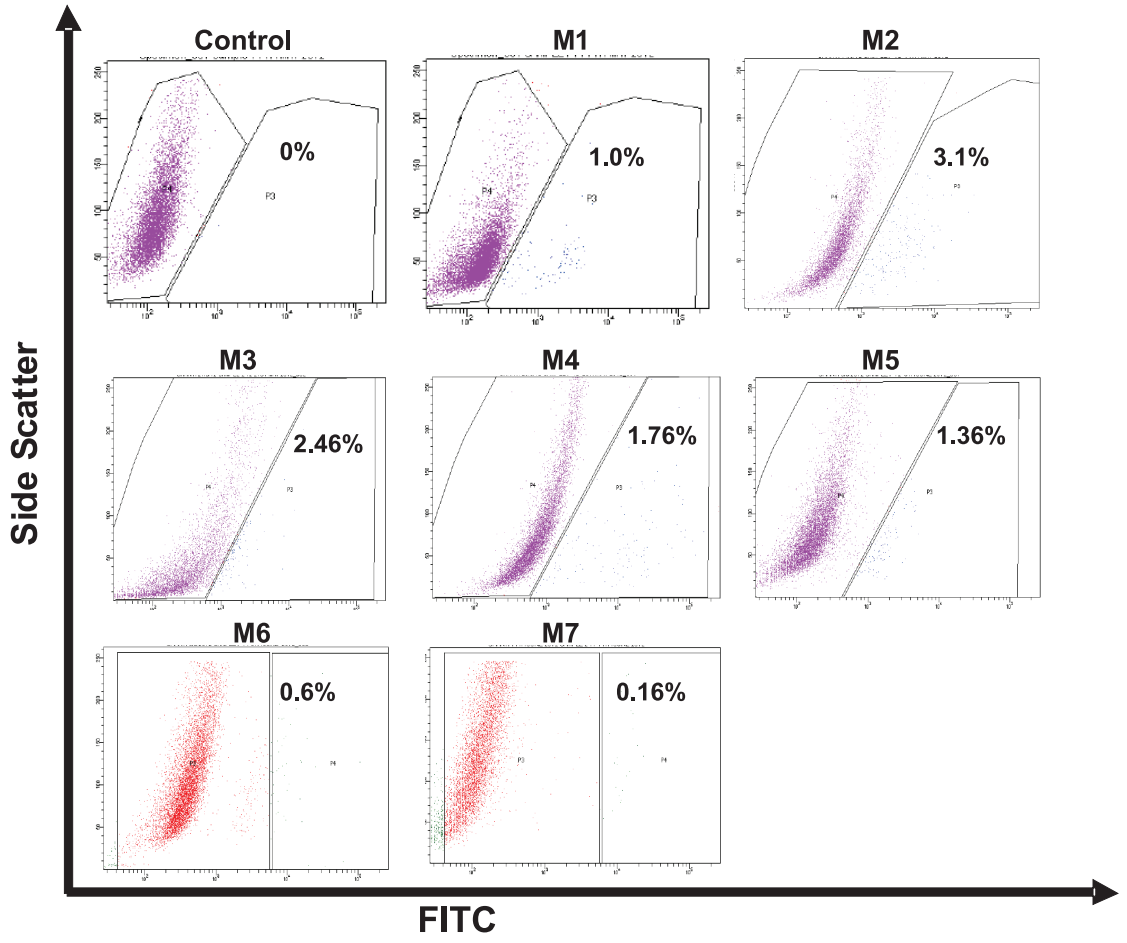


Fig. 1: Representative pictures of the set of dot plots showing the percentage of ALDH-positive breast (cancer) stem cells after serial passaging of mammospheres derived from the breast cancer cell line MCF7. Gating was done according to the individual DEAB controls.

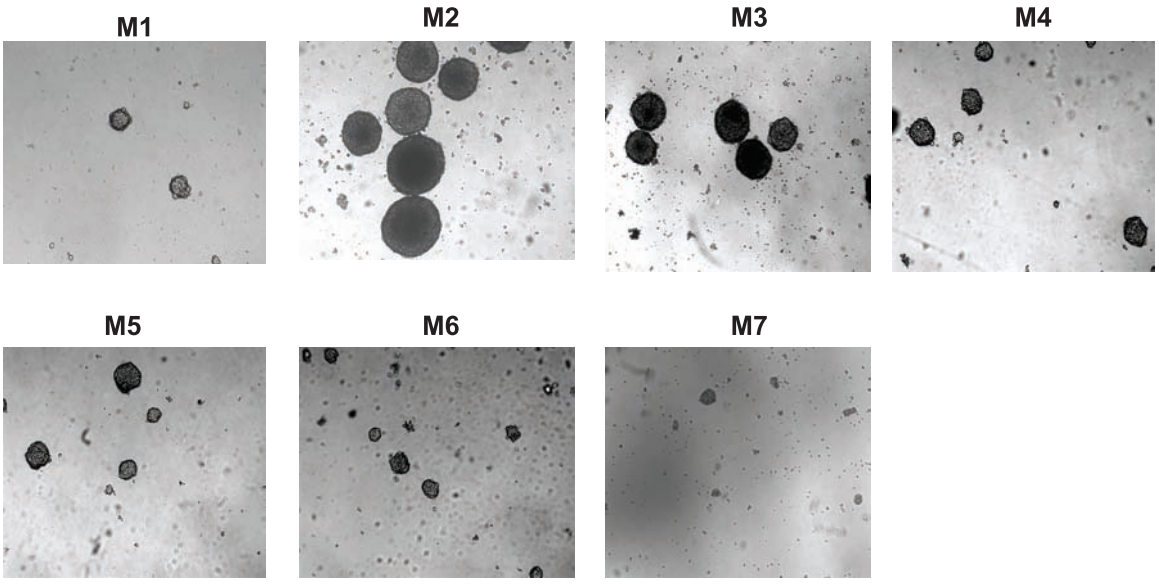


Fig. 2: Representative pictures of mammospheres formed by MCF7 after serial passaging. The mammosphere images of all the cell lines were captured using ImagePro software with a Nikon microscope at 4x magnification unless otherwise mentioned.

3. STUDY ON THE EFFECT OF siRNA MEDIATED ANDROGEN RECEPTOR GENE SILENCING ON ANDROGEN SIGNALING PATHWAY IN BREAST CARCINOMA

Scientific staff	**:** Dr. Sunita Saxena, Dr. Anurupa Chakroborty
Duration	**:** 2010-12

Aims, Objectives & Background

Androgen receptor (AR) is expressed in normal breast epithelial cells and in approximately 70 - 90% of invasive breast carcinomas. Androgen receptor expression is associated with a more favourable prognosis among women with estrogen receptor (ER)-positive tumors, but not among women with ER-negative tumors. In addition, AR positivity is associated with increased breast cancer mortality among women with triple-negative tumors.

There are important AR target genes that are activated or repressed by androgen bound AR. However, the underlying molecular mechanisms are poorly understood although genes repressed or activated by AR are key factors involved in cell proliferation, invasion or apoptosis. The androgen receptor (AR) has been reported to modulate TGFbeta1/Smad signaling and is, like transforming growth factor beta 1 (TGFbeta1) often overexpressed in hormone refractory prostate cancer (H G vander Poel 2005) whereas in human breast cancer cell lines, the role of AR in response to TGF beta signaling pathway has not been evaluated.

Hence, the objectives of the project are to assess AR-mediated transcription and cell growth in both AR (+) and AR (-) breast cancer cells through the knockdown of AR expression. Also, to analyze the expression of a panel of genes associated with important biological pathways like differentiation and development, cell cycle regulation and apoptosis or anti apoptosis, signal transduction like TGF beta pathway related to specific disease state like AR+/AR- breast carcinoma after knocking down androgen receptor gene and to examine the effect of anticancer drugs like CAF (Cyclophosphamide, Adriamycin, 5'Flurouracil) and Taxol on androgen receptor expression in breast cancer cells to find out whether CAF could inhibit cell proliferation through AR signalling pathway.

Work done during the year

qRT-PCR was performed in MDAMB 453 (AR+) and MDAMB231 (AR-) cell lines using an Human TGFß Signalling Targets PCR Array (*SuperArray Biosciences, Frederick, MD, USA*) as per manufacturer's instructions. The Human TGFß Signaling Targets PCR Array profiles the expression of 84 key genes responsive to TGFß signal transduction. Briefly, total RNA samples from MDA MB 453 (**Group 1**) and MDA MB 231 (**control**) breast cancer cells were isolated using RNeasy Micro Kit (*Qiagen*). An equal amount of RNA (1.5 µg) was used for reverse transcription using RT2 First Strand Kit (*SuperArray Biosciences*). PCR reactions were done using the RT2 profiler PCR array PAHS-235 (Human TGF beta Signalling Targets) on the ABI 7000 using RT2 Realtime SYBR Green PCR master mix. The total volume of the PCR reaction was 25 ml. The thermocycler parameters were 95ºC for 10 min, followed by 40 cycles of 95ºC for 15 s and 60ºC for 1 min. Five housekeeping genes were included on the array (B2M, HPRT1, RPL13A, GAPDH, and ACTB) to normalize the transcript levels. Results were analyzed as per user manual

guidelines using integrated web-based software package for the PCR Array System. All comparisons between groups were performed using two-tailed paired student's t-test. All P-values ≤0.05 were taken as significant.

The scatterplot was made to compare the normalized expression of every gene on the array between two groups by plotting them against one another to quickly visualize large gene expression changes (Fig. 1). The central line indicates unchanged gene expression.

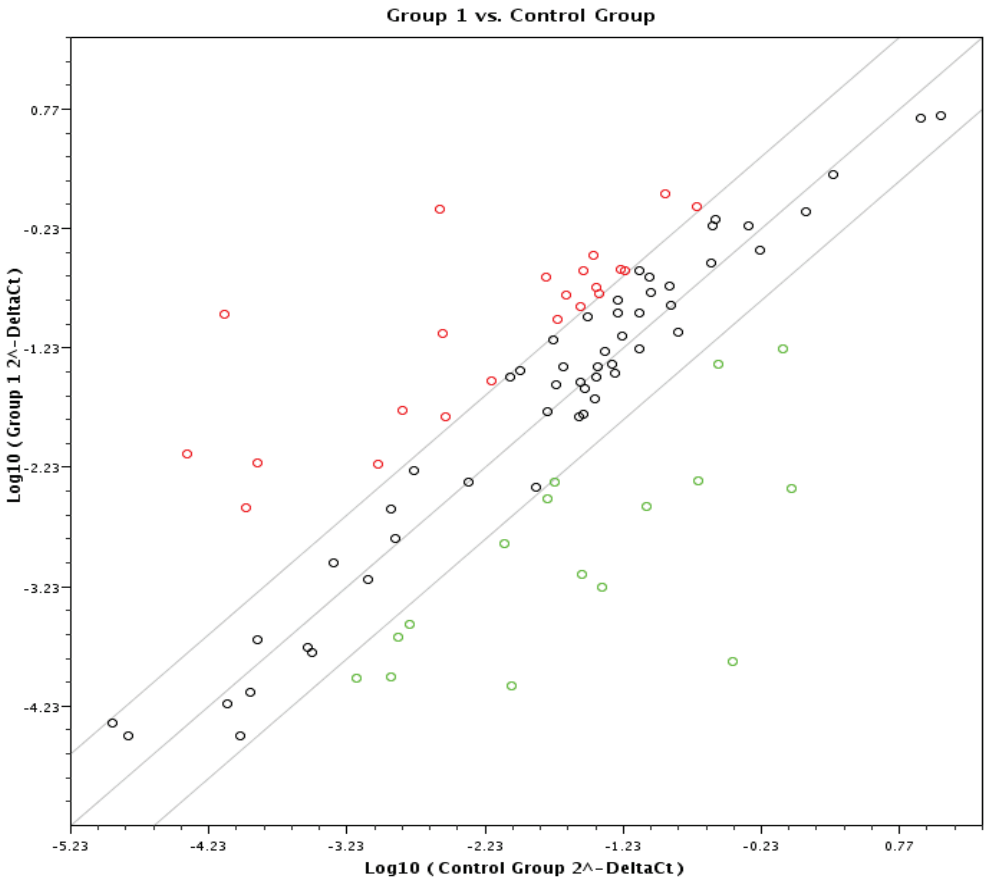


Fig. 1: The scatter plot showing the normalized expression of every gene on the array between two groups.

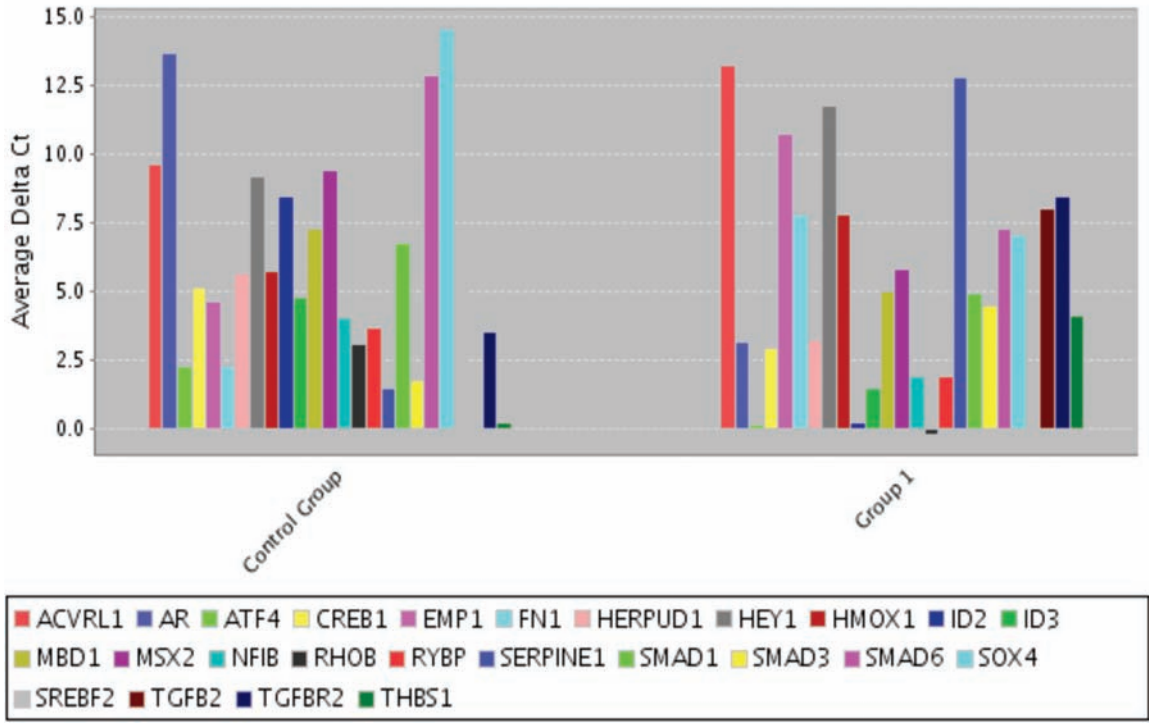


Fig. 2: Bar chart showing the expression change for a selected set of genes (≤ 0.05) across the tested groups.

Next, after the silencing of AR gene, mRNA expression of TGF beta signalling target genes in MDAMB 453 cells (AR+) will be analyzed through RT^2 PCR array to check the effect of AR silencing on TGF beta signalling pathway (Fig. 2).

URINARY BLADDER CANCER

1. CHARACTERISATION OF HOST IMMUNE PROFILE ASSOCIATED WITH PROGRESSION OF SUPERFICIAL TCC OF URINARY BLADDER BY MICROARRAY ANALYSIS

Scientific Staff	:	Dr. Sunita Saxena, Dr. Usha Agrawal, Dr. Sujala Kapur, Dr. Saurabh Verma, Ms Nitu Kumari
Name of Collaborator	:	Dr. N K Mohanty, Dr. Anup Kumar SJ hospital, New Delhi
Duration	:	2009-12

Aims, Objectives & Background:

Host immunity is known to play a role in cancer progression and a Th2 dominant state has been previously demonstrated in urinary bladder cancer. The progression and recurrence of tumors depends both on tumor cell markers and host factors. The identification of the mechanism of host anti-tumor immunity responsible for recurrences can help in identifying predictors of tumor behaviour as well as targets for immunotherapy. It would also help in understanding the biologic profile responsible both for recurrence and resistance to treatment. We have previously reported the dysregulation of cytokines in tumor tissue (Th2 upregulation in non-invasive and Th1 upregulation in invasive cancers) compared to normal adjacent mucosa of urinary bladder.

Work done during the year

Urinary and serum cytokines in urothelial cancer: Human cytokines, chemokines Bio-Plex Pro^{TM} 27-plex panel (*BIO-RAD*) kit including IL-1b, IL-1RA, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL-13, IL-15, IL-17, Eotaxin, FGF basic, G-CSF, GM-CSF, IFNG, IP-10, MCP-1, MIP-1a, PDGF-bb, MIP-1b, RANTES, TNF-a and VEGF was used for estimating cytokine concentration in urine and serum. Manufacturer provided standards were used to make the standard curve for each cytokine (Fig. 1).

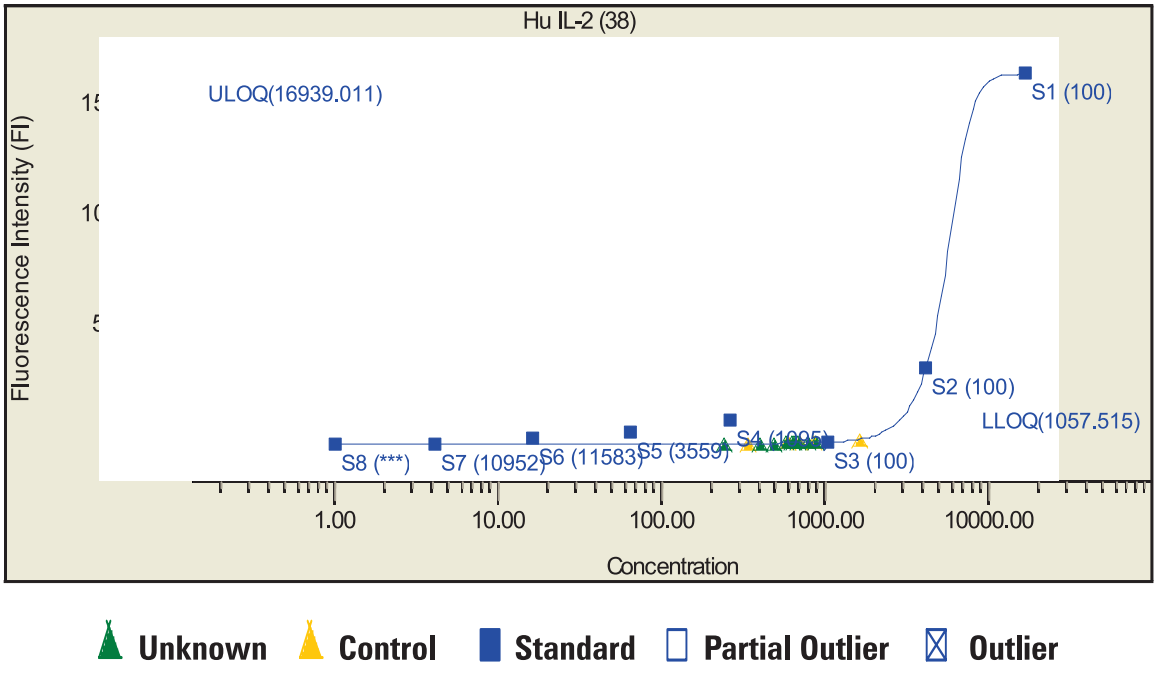


Fig. 1: Representative standard curve for IL-2 in multiplex cytokine assay.

Cytokines were estimated in duplicate in urine samples of 22 patients and 10 controls. Mann-Whitney U test was applied and showed significantly increased Th2 cytokine (IL-4), chemokines (IL-8, IP10) and MIP1b in urine of bladder cancer patients compared to healthy donors. In similar estimations in serum (34 patients and 10 controls), significant difference in cytokine concentration was not observed between patients and controls (Fig. 2).

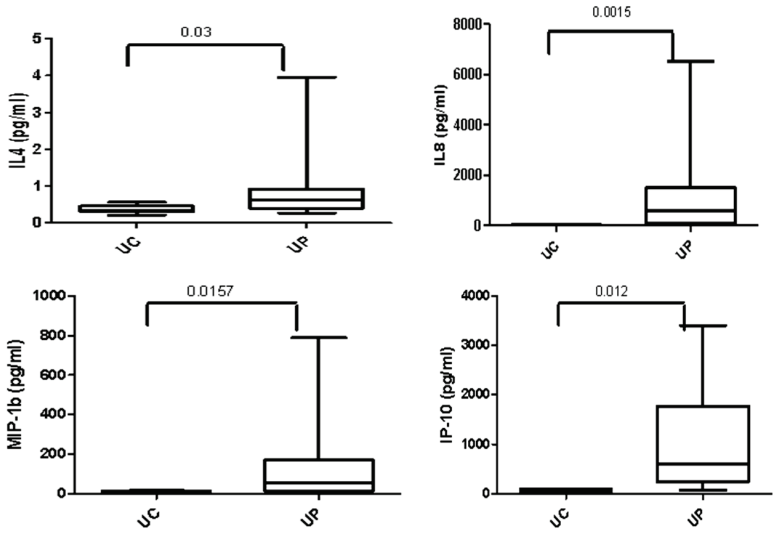


Fig. 2: Significantly upregulated urinary IL-4, IL-8, MIP-1b and IP-10 in urinary bladder cancer patients (UP) compared to healthy donors (UC).

Candidate gene expression in cytokines and NFκB pathway: Gene expression of IFNG (a Th1 representative molecule) and IL-4 (a Th2 representative molecule) in cytokine pathway, MYD88 and REL in NFκB pathway, regulatory factor CTLA4 and proinflammatory gene COX-2 were studied using Taqman-based Real time PCR on 80 tissue samples (51 bladder tumor and 29 adjacent mucosa). GAPDH was used as a housekeeping gene / endogenous control. Raw data were normalized and analysed by using ABI SDS software. REL, CTLA4 and COX-2 were found significantly upregulated (fold change > 2-fold; $p > 0.05$) in urinary bladder cancer compared to adjacent normal tissue (Fig. 3).

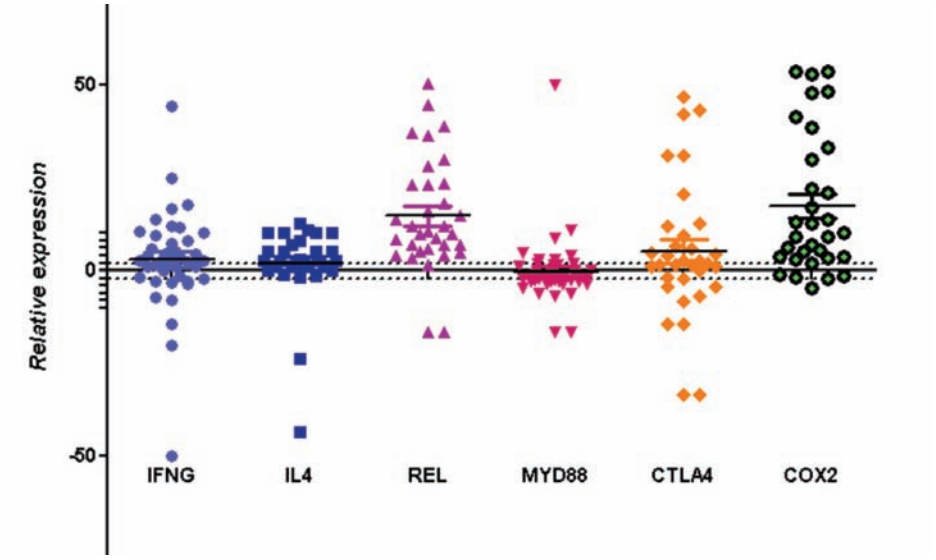


Fig. 3: Relative expression of candidate genes in urinary bladder cancer compared to adjacent bladder mucosa.

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

Scientific Staff	: Dr. Saurabh Verma, Dr. Usha Agarwal
Collaborators	: Dr. NK Mohanty, Safdarjung hospital, New Delhi
Technical Staff	: Mr. PD Sharma
Duration	: 2007-11

Aims, Objectives & Background

Cyclooxygenases catalyze the formation of prostaglandins from arachidonic acid and are upregulated in multiple types of solid tumors, including urinary bladder in humans. The cyclooxygenases also cause the release of prostaglandins which ultimately are responsible for inflammation, angiogenesis and tumor progression. 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. This appears as a rationale for the chemo-preventive effects of non-steroidal anti-inflammatory drugs in TCC. However, the reason of Cox-2 overexpression is not fully understood. In cell culture experiments, it has been found that Cox-2 is induced by inflammatory cytokines like IL-6 and IL-1beta. Based on this, we hypothesized an association between Cox-2 expression and expression of few proinflammatory cytokines like IL-1beta, IL-2, IL-6, TNF and TGF-beta. To test this hypothesis, we performed flow cytometric experiments for the respective antigens on the blood samples and studied the local responses in biopsy samples using IHC.

Work done during the year

We evaluated the role and expression pattern of cyclooxygenases and associated cytokines to understand the role of Cox-1 and Cox-2 in modulating cytokines and their effectors function in invasive and non-invasive TCC of human bladder.

Heparinized blood samples were collected from normal healthy individuals and TCC patients attending Urology Department, Safdarjung hospital for the study of Cox-1 and Cox-2 expression in association with inflammatory cytokines by flow cytometry.

The flow cytometry studies were done on PBMCs separated from the blood from 86 patients and 28 normal healthy individuals. Among 86 patients, 70 were non-invasive and 16 were invasive and 11.6%, 54.6% and 33.7% belonged to grade I, II and III respectively. Cox-2 expression was found increased in cancer patients in comparison to normal healthy individuals. A significant increase in mean percentage of IL-1beta and IL-6 expressing Cox-2 positive cells (42.78 ± 7.6 and 33.85 ± 6.57) was found in patients than normal controls (18.3 ± 8.7 and 23.08 ± 7.3). Significant variation in IL-1B ($p > 0.003$) and IL-6 ($p > 0.01$) level was observed in patients in comparison to normal group. Fig. 1 shows the dot plots with Cox-2 positive cells expressing IL-6 in control healthy subject and TCC patients. To corroborate our flow cytometric findings, we performed IHC and immunofluorescence experiments on biopsy samples from TCC patients and non-tumorous part of bladder adjacent to tumorous part from same patients for studying the local response.

Cox-2 expression was detected in cytoplasm of 49.1% and 86.2% of grade 2 and 3

malignant tumours respectively (Fig. 2) with increasing pattern from low grade to high grade tumours. Cox-2 was not detected in histologically normal non-tumorous bladder epithelia.

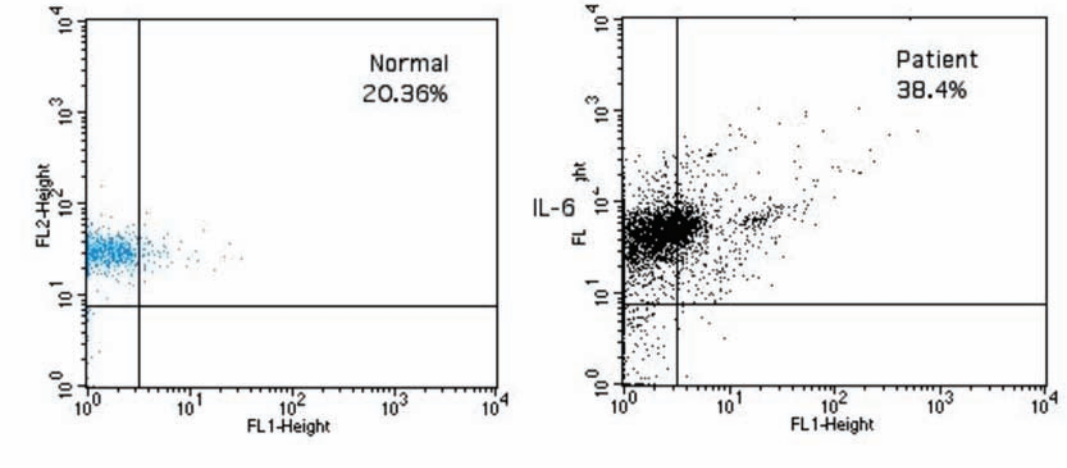


Fig. 1: Dot Plot showing the Cox-2 positive cells IL-2 expression.

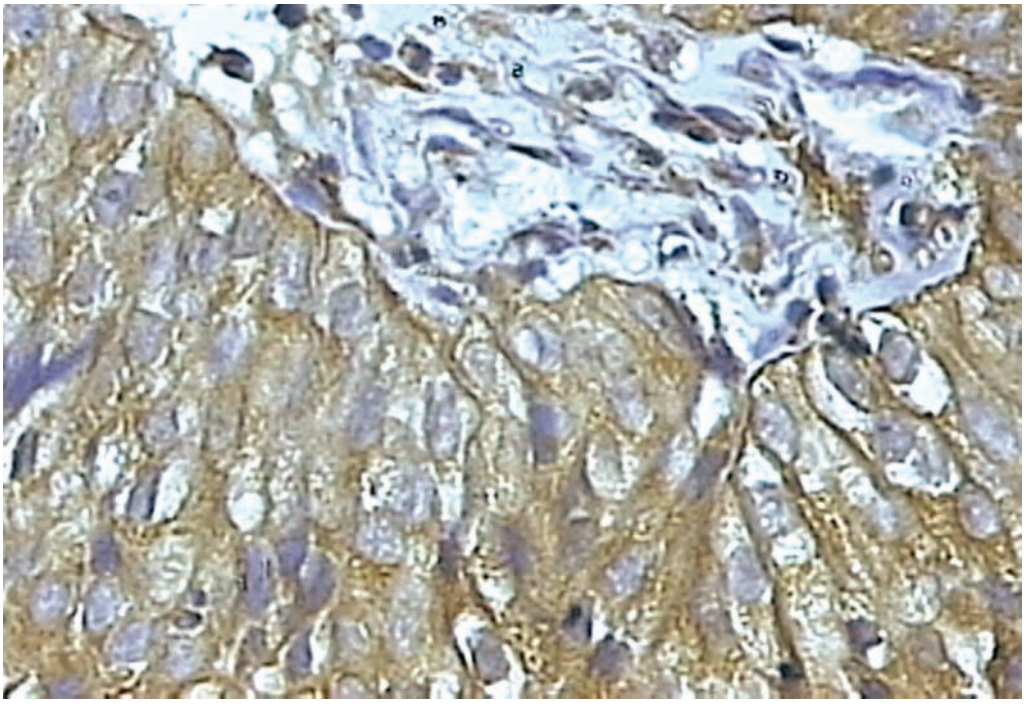


Fig. 2: Tumour cells showing the positivity of Cox-2 in TCC patient.

Cancers in North-East India

1. EFFECT OF TOBACCO AND PESTICIDE IN CANCERS IN NORTH-EAST INDIA

Scientific Staff	:	Dr. Sunita Saxena, Dr. Sujala Kapur, Mr. Dhirendra Yadav, Ms Rakshan Ihsan, Ms Thoudam Regina
In Collaboration with	:	Dr. AC Kataki, Dr JD Sharma, (Dr. BB Cancer Institute, Guwahati, Assam)
		Dr. RK Phukan, Dr. J Mahanta (RMRC, Dibrugarh, Assam)
Duration	:	2005-11

A. Oral Cancer

Oral cancer is a multi-factorial disease and influenced by both aetiological factors and ethnicity; as a result molecular profiles of oral cancer vary throughout the world. Altered gene expression profiling may be attributed to several types of genetic alterations such as base changes, insertions, deletions, chromosomal translocations, inversions, loss of heterozygosity and copy number variations. In the current study, targeted re-sequencing of 169 functionally relevant and potentially important genes was done to analyze mutational changes. In our study, 4837 exon regions of interest were analyzed with average read depth of 47.14. A large number of mutational changes were observed including 96 SNPs (50 novel and 46 known SNPs) and 46 InDels (29 novel and 17 known InDels). Most of the known SNPs with high read depths (> 50) were located in tumor suppressor genes in addition to some SNPs in non-coding regions. GO analysis is being done to understand various biological functions of these genes that may have possible relevance in the carcinogenic mechanism of oral cancer.

B. Lung Cancer

Complex disease such as cancer results from interactions of multiple genetic and environmental factors. Studying these factors singularly cannot explain the underlying

pathogenetic mechanism of the disease. Multi-analytical approach, including logistic regression (LR), classification and regression tree (CART) and multifactor dimensionality reduction (MDR), was applied in 188 lung cancer cases and 290 controls to explore high order interactions among xenobiotic metabolizing genes and environmental risk factors. Smoking was identified as the predominant risk factor by all three analytical approaches. Individually, CYP1A1*2A polymorphism was significantly associated with increased lung cancer risk (OR= 1.69; 95% CI= 1.11–2.59, p= 0.01), whereas EPHX1 Tyr113His and SULT1A1 Arg213His conferred reduced risk (OR= 0.40; 95% CI= 0.25–0.65, p= 0.001 and OR= 0.51; 95% CI= 0.33–0.78, p= 0.002 respectively). In smokers, EPHX1 Tyr113His and SULT1A1 Arg213His polymorphisms reduced the risk of lung cancer, whereas CYP1A1*2A, CYP1A1*2C and GSTP1Ile105Val imparted increased risk in non-smokers only. While exploring non-linear interactions through CART analysis, smokers carrying the combination of EPHX1 113TC (Tyr/His), SULT1A1 213GG (Arg/Arg) or AA (His/His) and GSTM1 null genotypes showed the highest risk for lung cancer (OR= 3.73; 95% CI= 1.33–10.55, p= 0.006), whereas, combined effect of CYP1A1*2A 6235CC or TC, SULT1A1 213GG (Arg/Arg) and betel quid chewing showed maximum risk in non-smokers (OR= 2.93; 95% CI= 1.15–7.51, p= 0.01). MDR analysis identified two distinct predictor models for the risk of lung cancer in smokers (tobacco-chewing, EPHX1 Tyr113His and SULT1A1 Arg213His) and non-smokers (CYP1A1*2A, GSTP1 Ile105Val and SULT1A1 Arg213His) with testing balance accuracy (TBA) of 0.6436 and 0.6677 respectively. Interaction entropy interpretations of MDR results showed non-additive interactions of tobacco chewing with SULT1A1 Arg213His and EPHX1Tyr113His in smokers and SULT1A1 Arg213His with GSTP1 Ile105Val and CYP1A1*2C in non-smokers. These results identified distinct gene-gene and gene environment interactions in smokers and non-smokers, which confirms the importance of multifactorial interaction in risk assessment of lung cancer.

C. Gastric Cancer

In our earlier study on gene expression profiling, we have found genes responsible for apoptosis, inflammatory response, immune response, angiogenesis, cell migration and cell proliferation to be significantly deregulated. Genes such as *LDHB*, *PXN*, *RYK*, *RET*, *ANG*, *BMP7*, *RAD51L3* and *CXCL10* involved in these biological processes were analyzed by real time PCR. Further genomic alteration in gastric cancer is being analyzed by NGS on Solexa plateform.

The total number of SNPs observed were 178 in cases whereas those of indels were 23 (12 insertion and 11 deletion). Most of the observed SNPs were known SNPs, however, novel SNPs were also found in the coding regions of genes that may have functional relevance in the pathogenesis of gastric cancer. This study is continuing and data is being analyzed.

Future Plan of Work

Study will be completed after data analysis.

2. GENOME-WIDE ANALYSIS OF GENETIC ALTERATIONS IN PATIENTS WITH OESOPHAGEAL CANCER FROM NORTH-EAST INDIA USING SINGLE NUCLEOTIDE POLYMORPHISM ARRAYS

Scientific Staff	:	Dr. Sujala Kapur, Dr. Sunita Saxena, Mr. Ashish Bhushan
In Collaboration with	:	Dr. AC Kataki, Dr. JD Sharma, Dr. BB Barthakar, Dr. A Rai (Dr. BB Cancer Institute, Guwahati, Assam)
		Dr. RK Phukan, Dr. J Mahanta (RMRC, Dibrugarh, Assam)
Duration	:	2011-12

Aims, Objectives & Background

Oesophageal cancer is sixth leading common cause of death from cancer worldwide. In India, it is 4th most common with high incidence and mortality rated by GLOBOCAN 2008 (http://globocan.iarc.fr/). In particular, the highest incidence of this cancer in India has been reported from north-east region. This is second leading cancer in men and third leading cancer in women in north-east population. The major factors identified for oesophageal cancer are nutritional deficiencies, intake of pickled vegetables, low socioeconomic status and major environmental risk like alcohol consumption, smoking and tobacco. In north-east population, these factors are highly relevant cause of oesophageal cancer. It has been also reported earlier that chewing of fermented areca

nut with or without tobacco is independently associated with development of cancer in this population.

In Assam region of north-east India, familial aggregation of esophageal cancer has been reported alongwith genetic susceptibility, in combination with exposure to environmental risk factors (tobacco paste and tobacco water). Single nucleotide polymorphism in genes that are critically involved in cell apoptosis, cell cycle control, cell differentiation, cell, invasion, migration, or other functions may also explain the pathogenesis of oesophageal cancer. Currently, the genetic and environmental risk factor that may be responsible for the high prevalence and family aggregation of oesophageal cancer in north-east India remains unclear. Our aim is to investigate the chromosomal instability profile and characterization of genetic factors associated with the development and progression of oesophageal cancer and identification of biomarkers for susceptibility / familial clustering in high risk region of India. In addition, functional role of key genes on established oesophageal cancer cell lines by siRNA knockdown will also be studied.

Work done during the year

Sample collection and genomic DNA extraction

28 pairs of endoscopy biopsy and their adjacent normal tissue specimens (in RNA later solution), 5 ml of peripheral blood (in EDTA) were collected from Dr. Bhubaneshwar Borooah Cancer Institute (BBCI), Guwahati, Assam and stored at –70^{o} C.

For preliminary study, five tumor tissues and blood samples (germ line DNA) from the same patients have been processed (Table 1).

Table 1: Sample Processed for study

Sl. No.	Patient detail		Clinical	Consumption Habit				Food Habits				
	Sample ID.	Sex / Age	Morphology	AH	BQH	SH	TH	Veg	Egg	Fish	Chicken	Red meat
1	GW/EC/004/11	M/56	MDSCC	YES	YES	YES	NO	NO	YES	YES	YES	YES
2	GW/EC/005/11	M/55	MDSCC	NO	YES	YES	YES	NO	YES	YES	YES	YES
3	GW/EC/014/11	F/45	MDSCC	NO	YES	NO	NO	NO	YES	YES	YES	NO
4	GW/EC/032/11	M/62	MDSCC	YES	YES	YES	NO	NO	YES	YES	NO	NO
5	GW/EC/042/11	M/62	MDAC	YES	YES	NO	YES	NO	YES	YES	NO	YES

MDSCC - Moderately Differentiated Squamous Cell Carcinoma,
AH - Alcohol Habit, **BQH** – Betel Quid Habit, **SH** – Smoking Habit, **TH** – Tobacco Habit, **Red Meat** – Beef/Pork/Mutton

All tumor samples were moderately differentiated squamous cell carcinoma. All patients had betel quid chewing habit. DNA was extracted using the *Qiagen* Qlamp DNA Mini kit, and diluted to concentration of 50 ng/µl using reduced TE buffer (Fig. 1).

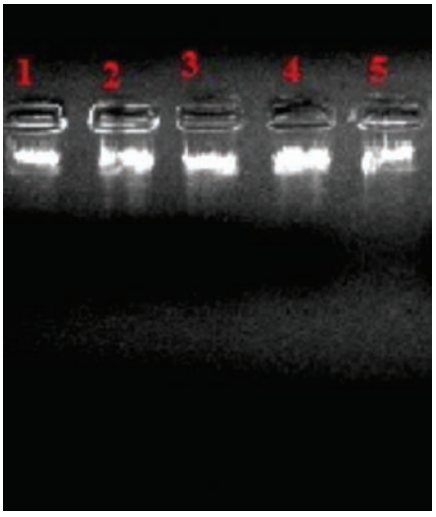


Fig 1: Agarose gel electrophoresisis showing genomic DNA

Affymetrix® Genome-Wide Human SNP Array 6.0

For further genome wide analysis for chromosomal aberration and SNPs, the genomic DNA plate will be processed using Affymetrix GeneChip Human Mapping 6.0. Affymetrix® Genome Wide Human SNP Array 6.0 contains 1.8 million markers, including 946,000

probes for the detection of copy number variants and 906,600 SNPs. SNPs on the array are present on 200 to 1,100 base pairs (bp) Nsp I or Sty I digested fragments in the human genome, and will be amplified using the Genome-Wide Human SNP Nsp/Sty Assay Kit 6.0.

The following method or analysis on Affymetrix® Genome-Wide Human SNP Array 6.0 was included Stage 1: Sty I Restriction Enzyme Digestion, Stage 2: Sty I Ligation, Stage 3: Sty PCR, Stage 4: Nsp I Restriction Enzyme Digestion, Stage 5: Nsp I Ligation, Stage 6: Nsp I PCR, Stage 7: PCR Product Pooling and Purification, Stage 8: Quantitation, Stage 9: Fragmentation, Stage 10: Labeling Stage 11: Target Hybridization.

Briefly, total genomic DNA will be digested with Nsp I and Sty I restriction enzymes and ligated to adaptors that recognize the cohesive 4 bp overhangs. All fragments resulting from restriction enzyme digestion, regardless of size will be substrates for adaptor ligation. A generic primer that recognizes the adaptor sequence was used to amplify adaptor-ligated DNA fragments. PCR conditions had been optimized to preferentially amplify fragments in the 200 - 1,100 bp size range. PCR amplification products for each restriction enzyme digest will be combined and purified using polystyrene beads.

The amplified DNA will then be fragmented, labelled and hybridized to a Genome-Wide Human SNP Array 6.0. Hybridization was done at 48˚C for 16-18 hours at 60 rpm in the hybridization oven. The arrays will be washed and stained by incubation with streptavidin, then biotinylated antistreptavidin, followed by streptavidin-R-phycoerythrin conjugates using the Affymetrix Fluidics Station 450. Finally, the microarrays will be scanned in the Affymetrix GeneChip Scanner 3000 using GeneChip Operating System 1.1.1 with Patch 5 or higher (GCOS, Affymetrix). The data will be analyzed by GeneChip® Operating Software. The study is continuing.

3. IMMUNOGENETIC PROFILE OF NASOPHARYNGEAL CANCER IN A HIGH-PREVALENCE REGION OF NORTH-EAST INDIA

Scientific Staff	:	Dr. Sujala Kapur, Dr. LC Singh, Dr. Saurabh Verma, Dr. AK Mishra, Ms Meena Lakhanpal, Dr. Sunita Saxena
In Collaboration with	:	Dr. AC Kataki, B. Barooah Cancer Institute, Guwahati, Assam Dr. Y Mohan Singh, RIMS, Imphal (Manipur)
Project Coordinator	:	Dr. Sunita Saxena
Duration	:	2010-13

Aims, Objectives & Background

Nasopharyngeal Carcinoma (NPC) unlike other head and neck cancers shows a clear regional and racial prevalence. The incidence of NPC is high in the southern region of China, south-east Asia, Alaska and native Greenlanders. A high incidence of NPC in North-East (NE) region of India has been reported where it is the eighth most common cancer. The differences in geographic and ethnic distribution reflect the multifactorial etiology of NPC, including the Epstein-Barr virus (EBV) infection, ethnics, genetic susceptibility, environmental factors and food consumption. NPC is a squamous epithelial cancer arising from the lateral wall surface of nasopharynx, however, it is one of the most confusing, commonly misdiagnosed and poorly understood disease. The project was undertaken with the following aims:

1. To study the allelic variation in HLA class I and class II loci in patients with nasopharyngeal carcinoma as compared to the control population from north-east India and analyze if there is a correlation of specific HLA alleles to disease susceptibility in this region.

2. To study if there is any variation at the genetic level in HLA class III region that contains genes encoding the pro-inflammatory cytokines (TNF-α and TNF-β) and heat shock proteins HSP, gp 70.

3. To analyze if the presence of Epstein Barr viral sequences in the tumor tissue can explain the high prevalence of nasopharyngeal carcinoma in this region.

Work done during the year

During the year, 91 blood samples from newly diagnosed cases of NPC have been collected. Of these, 37 are from Guwahati and 54 are from Imphal. 35 tissue samples from these 91 cases have also been collected. Genomic DNA was extracted from 55 whole blood samples and 55 controls (Table 1).

Table1: Blood and tissue samples received from the two centres of north-east India.

Centres	Blood Samples received		Tissue Samples received	
	Cases	Controls	Cases	Controls
RIMS, Imphal	54	43	07	1
BBCI, Guwahati	37	33	28	-
Total	**91**	**76**	35	1

This study aims to do the genotyping of the HLA region at 6p21.3 using 33 polymorphic microsatellite markers. These markers have previously been identified by the use of sequence data from the US National Center for Biotechnology Information map.

Of the 33 markers that will be analyzed in this study, standardization of PCR conditions for 31 microsatellite markers has been done. The primers used were synthesized using FAM-labelled forward primer and unlabelled reverse primer from *M/s Biolink (India)* with various concentrations of $MgCl_2$ (1.5mM, 1.8mM, 2.0mM 2.5mM, 3mM and 3.5mM) at gradient temperatures as shown in Fig.1.

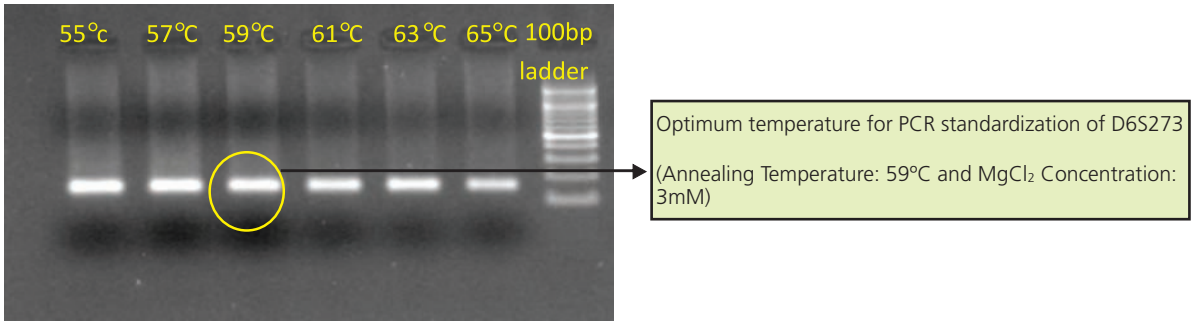


Fig. 1: Standardization of PCR conditions for microsatellite marker D6S273

Using standardized conditions, the amplification of microsatellite markers in genomic DNA extracted from whole blood of 55 cases of nasopharyngeal cancer and 55 controls was done. Quality and purity of PCR product was estimated by agarose gel electrophoresis containing ethidium bromide (Fig. 2).

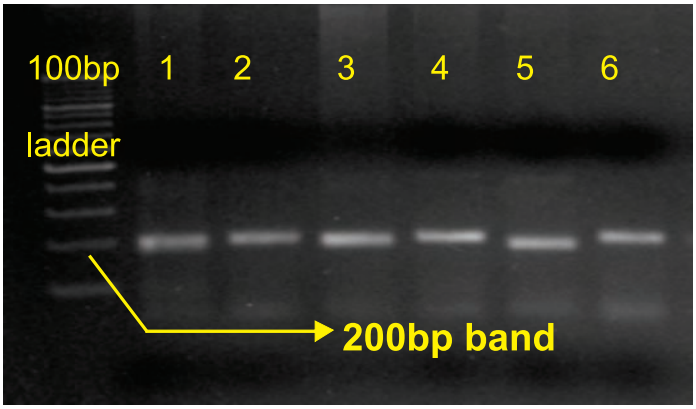


Fig 2: Agarose gel showing PCR amplification of microsatellite marker D6S2699 in 6 different samples of north-east India. The size of the band was expected around 181-199 bp.

Following the confirmation of amplification in all the 55 samples, samples were prepared for fragment length analysis in a DNA sequencer. For this, a master mix containing GeneScan-500 LIZ Size Standard (*Applied Biosystems*), Hi-Di formamide per sample was prepared and aliquoted into each well of 96-well optical reaction plate (*Applied*

Biosystems). FAM-labelled PCR amplicon from each sample was loaded into its well. The capillary electrophoresis was performed using the *Applied Biosystems ABI PRISM 3130xl* Genetic Analyzer with Data Collection Software Version 2.0. The length of the fluorescently (6 FAM) labelled fragments was determined with Gene Scan Analysis Software (Gene Mapper version 4.0) (*Applied Biosystems, Foster City, CA*) (Fig. 3).

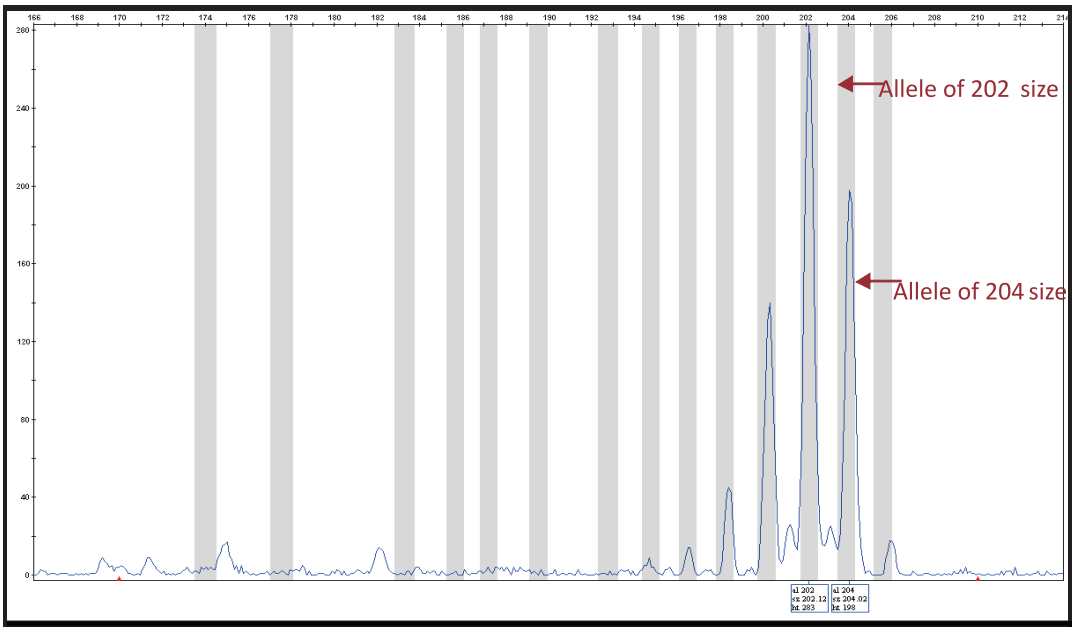


Fig. 3: Genotyping for D6S2673 analyzed by gene mapper software; this sample shows heterozygous alleles of 202 and 204. sizes.

Analysis of preliminary results was done using Gene Mapper software. It showed many allelic variants for the all the 31 micosatellite markers.

Detection of EBV

a) By PCR

In the current project, molecular methods are being used to detect EBV in tissue / blood samples using PCR and *in situ* hybridization. During the year, to standardize the PCR conditions and to detect EBV EBNA2 gene variant, EBV-positive cell line B95.8 (marmoset blood leukocytes EBV transformed cell line that releases high titers of transforming EBV)

was obtained from ATCC. Conventional PCR method was applied in both cases and control samples to standardize the optimum PCR conditions for detection of EBV. In addition, another cell line which is also EBV-positive (Raji cell line) was also obtained and processed for standardization of PCR to detect EBV.

Cell culture for B95.8 cell line: The B95.8 cell line was grown at 37°C in 5% carbon dioxide (CO_2) incubator and cultured with complete RPMI 1640 medium (*GIBCO, USA*) supplemented with 2mM L-glutamine, 100 IU/ml penicillin, 100 IU/ml streptomycin, 2.0 g/L sodium bicarbonate (*Sigma, USA*) and 10% Foetal Bovine Serum (FBS) (*GIBCO, USA*). The cells were grown until 70-90% confluence before being harvested.

Cell culture for Raji cell line: The Raji cell line was cultured in RPMI 1640 (*GIBCO, USA*) medium as a suspension culture at 37^0C in CO_2 incubator. The CO_2 concentration in the incubator was maintained at 5%. The RPMI 1640 medium was supplemented with 2 mM L-glutamine, 100 IU/ml penicillin, 100 IU/ml streptomycin (*GIBCO, USA*), 1.5 g/L sodium bicarbonate (*Sigma, USA*), 4.5 g/L glucose (*Sigma*), 10 mM HEPES (*Sigma*) and 10% Foetal Bovine Serum (FBS) (*GIBCO, USA*). The cells were grown until 70-90% confluence before being harvested.

The primers were synthesized from *M/s Biolink (India)* and were standardized with various concentrations of $MgCl_2$ (1.5mM, 1.8mM, 2.0mM 2.5mM, 3mM and 3.5mM) at gradient temperatures as shown in Fig. 4.

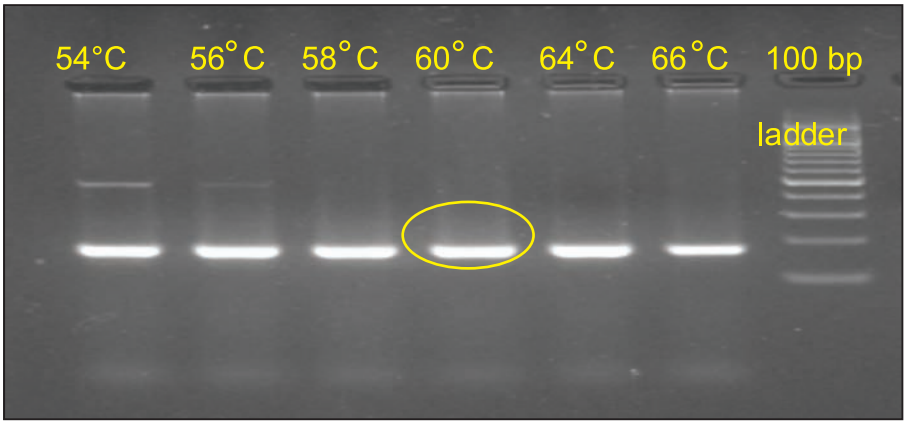
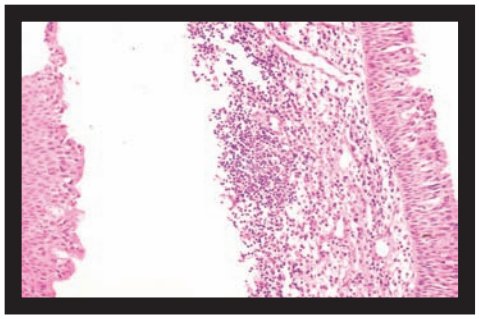


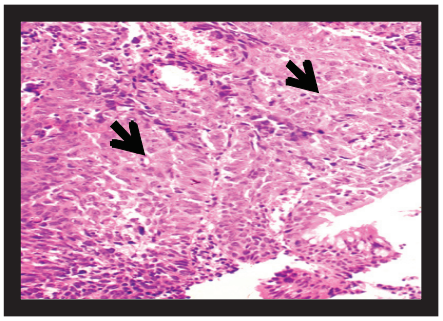
Fig. 4: Optimum temperature for PCR standardization of EBNA2 (annealing temperature: 60°C and MgCl2 concentration: 1.8mM

b) By In Situ Hybridization:

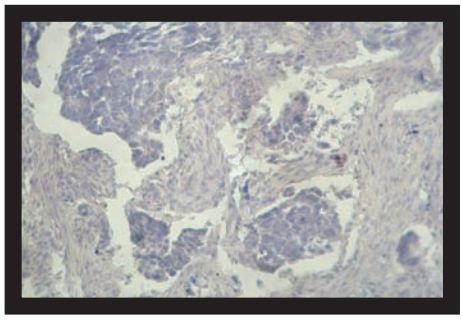
Epstein-Barr virus (EBER) PNA probe / fluorescein was obtained from *DAKO* and used for the detection of EBV infection by *in situ* hybridization. For this formalin-fixed, paraffin-embedded tissue sections were processed as per the manufacturer's instructions. Briefly, hybridization was followed by a post-hybridization wash with a stringent wash solution. This was followed by incubation with alkaline phosphatase-conjugated rabbit F(ab') anti-FITC substrate (enzyme substrate, BCIP / NBT) at room temperature followed by a wash. Finally the specimen was mounted. Positive staining was recognized under light microscope as a dark blue / black colour at the site of hybridization (Fig. 5).



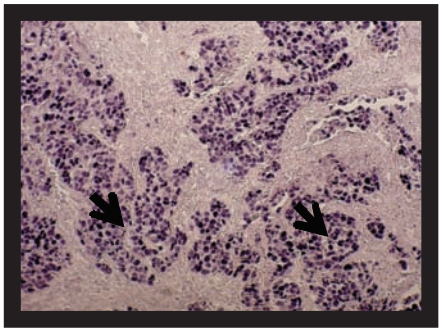
A) H&E - Normal epithelial cells in nasopharyngeal biopsy



B) H&E – Tumour Cells in nasopharyngeal biopsy



C) ISH – Tumour cells with no hybridization for EBER probe



D) ISH – Tumour cells with nuclear positivity, dark blue/black colour at the site of hybridization with EBER probe

Fig. 5: Images showing standardization of conditions for *in situ* hybridization and H&E staining in paraffin

Future Work Plan

Microsatellite Study: DNA isolation will be continued in all samples that will be received further. Standardization of PCR for the remaining 2 microsatellite markers will be done by using various concentrations of $MgCl_2$ and gradient temperatures. This will be followed by microsatellite study in the remaining patients and control samples for all the 33 microsatellite markers.

Detection of EBV: Both PCR amplification as well as *in situ* hybridization (in tissue samples of patients) will be done for EBV detection in patient and control blood samples.

Hematopoietic-Lymphoid Malignancies

1. GENETIC AND EPIGENETIC PROFILE OF ACUTE MYELOID LEUKEMIA

Scientific staff	:	Ms Asheema Khanna, Dr. Sujala Kapur
In collaboration with	:	Dr. Sumita Saluja, Safdarjung hospital
Duration	:	2010-13

Aims, Objectives & Background

Classic genetics alone cannot explain the diversity of phenotypes within a population. Epigenetic phenomena are required for proper development and cellular differentiation within normal tissues. Epigenetics is defined as modifications of DNA or associated factors that have information content and are heritable. There are potential links between epigenetics and cancer. Recent evidence suggests that AML is also associated with epigenetic changes in hematopoietic progenitor cells that alter normal mechanisms of cell growth, proliferation and differentiation. Understanding the contribution of epigenetic molecular mechanisms and biological pathways involved in AML or subtype, may help in developing epigenetic biomarkers. Correlating non- expression silenced genes with biologic / clinical outcomes may lead to a greater understanding of leukemogenesis and improve treatment and possible model for the interplay between environment and human epigenome. There are just a few reports that have been used to suggest inheritance of epigenetic states and the molecular mechanisms and biological pathways in the pathogenesis of AML. Unlike genetic alterations, epigenetic changes are potentially reversible.

The current study has been designed with the objective to characterize the epigenetic modifications associated with AML and if there is any correlation between epigenetic modifications and cytogenetic abnormalities.

This study is being conducted on patients with a confirmed diagnosis of Acute Leukemia

(AL) who are admitted to the Department of Hematology, Safdarjung hospital, New Delhi for induction chemotherapy.

Work done during the year

Flow cytometric immunophenotyping was done in all 52 samples to diagnose Acute Leukemia (AL) as Acute Lymphocytic Leukemia (ALL) and Acute Myeloid Leukemia (AML). Of these, 14 samples of B-ALL, 11 samples of T-ALL and 27 samples of AML have been diagnosed. Cytogenetic status of the samples is shown in Table 1.

Table 1: Cytogenetic status of the cases

Type of Translocation	No. of Samples (N= 52)	Percentage
PML-RARA	3	6%
AML-ETO	4	8%
MLL-AF9	2	4%
OTT-MAL	1	2%
Normal cytogenetics	40	76%
Cytogenetics status not defined	2	4%

Mononuclear cells (Blasts) were isolated from bone marrow and peripheral blood samples of pediatric and adult AML patients. These cells were cultured for short term with RPMI 1640 and with or without foetal bovine serum for up to 72 hrs. Cells were harvested and stored in liquid nitrogen. These cells will be used to understand methylation patterns in leukemia cells.

To understand the cell cycle regulation, we have standardized cell cycle analysis using PBMCs from 5 AML patients. DNA analysis was performed by staining the cells with propidium iodide, a fluorescent dye which intercalates between DNA base pairs. The fluorescent intensity of the dye within the nucleus is directly related to the amount of DNA present. The purpose of cell cycle analysis was to study the effect of epigenetics on cell cycle stages. Acquired data was analyzed by Flowjo Software. Results are shown in Figs. 1-2.

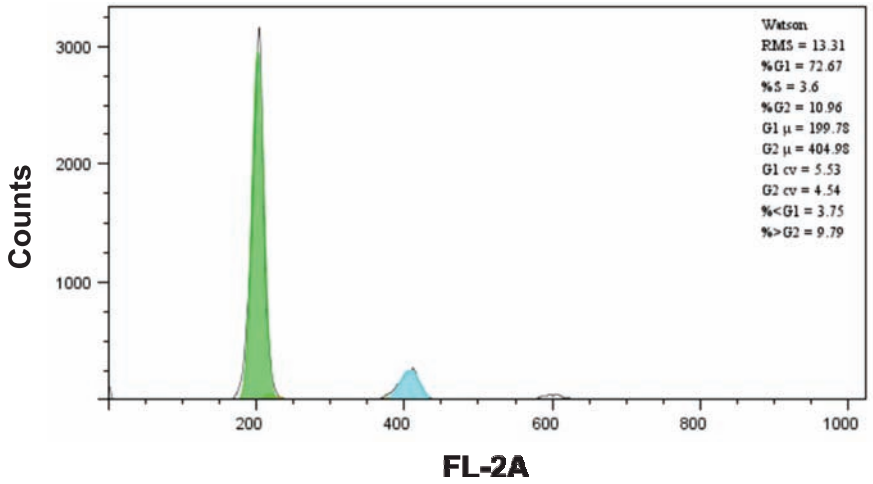


Fig. 1: Representative figure of cell cycle analysis of sample of acute myeloid leukemia

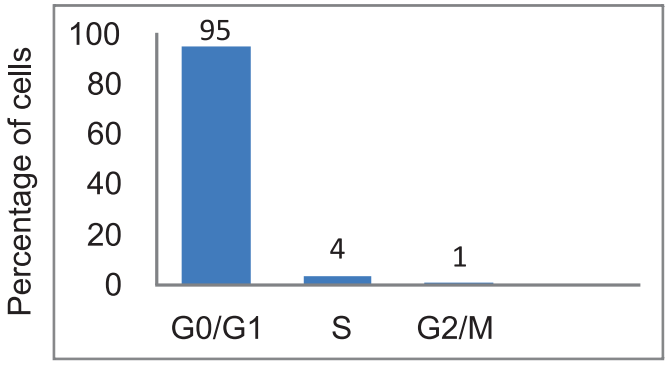


Fig. 2: Cell Cycle analysis of AML, mean % of total cells in G1/G0, S and G1/M phase (n=5).

2. REDOX REGULATION OF LYMPHOCYTE SIGNALING IN B-CLL

Scientific Staff	: Dr. Anand Kumar Verma, Dr. Sujala Kapur
In collaboration with	: Dr. Sumita Saluja, Safdarjang hospital, New Delhi
Duration	: 2009-12

Aims, Objectives & Background

Chronic Lymphocytic Leukemia (CLL) is an abnormal neoplastic proliferation of mature B-cells which originates in the bone marrow and develops in the lymph nodes. The cells accumulate mainly in the bone marrow and blood.

The pathogenesis of B cell chronic lymphocyte leukemia (B-CLL) is still not very clear. The role of reactive oxygen species (ROS) in the activation and proliferation of B-cells leading to a longer survival of B cells in B-CLL is unknown. In addition, the role of ROS in causing the dysfunction of T cells in B-CLL is also not known.

The aims of the present study are:

- To investigate the role of redox in B-cell activation, proliferation and survival in B-CLL.
- To investigate the role of redox on co-stimulatory and co-inhibitory molecules involved in T- and B-cell communication in B-CLL.

Work done during the year

Analysis of effect of redox on B cell activation in B-CLL:

To study the effect of ROS in B cell activation, 20 samples of healthy human PBMCs and B-CLL PBMCs were analysed using CD80 as an activation marker for B cell activation. Cells were cultured in different conditions including pokeweed mitogen as control to study the role of redox. Data shown are mean of 20 cases of BCLL patients (Fig. 1).

There was significant increase in activation of CLL B cells in comparison to age and sex-matched control samples. The results show that ROS generation can lead to antigen independent activation of B cells. However, B cell activation in these cells does not lead to antibody production. The molecular mechanism involved in ROS generated B-CLL activation needs to be further explored.

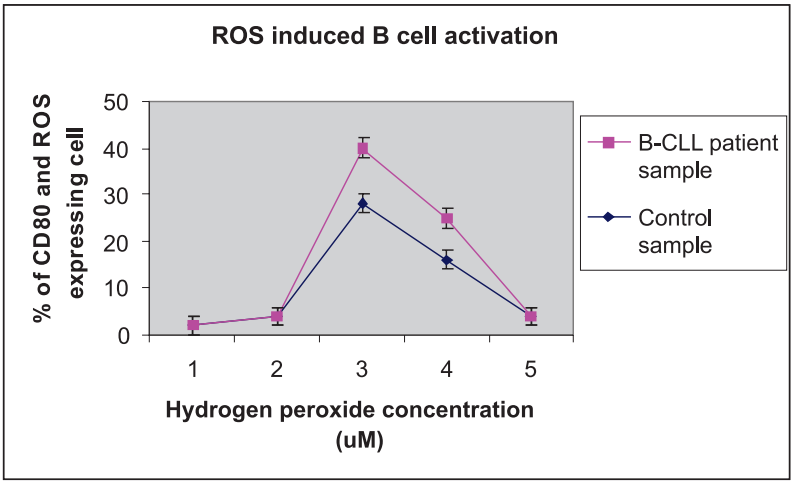


Fig. 1: Represents B cell activation in response to exogenous ROS. Data are mean values of 20 samples of CLL patients and control samples.

Expression analysis of co-stimulatory and co-inhibitory molecules in B-CLL:

To study the role of co-stimulatory molecules, B cells (CD19+CD5+) and CD3+ T cells were purified from B-CLL PBMC and cultured in conditioned media for 96 hours. Cells were induced by treating with optimized concentration of hydrogen peroxide to generate exogenous ROS. The expression of co-stimulatory and co-inhibitory molecules was measured using flow cytometry. The cells of B-CLL samples were found to enhance the expression of co-stimulatory and co-inhibitory molecules on induction as compared to controls. The expression of co-stimulatory molecules is shown in Figs. 2-5 and expression of co-inhibitory molecules is shown in Figs. 6-7.

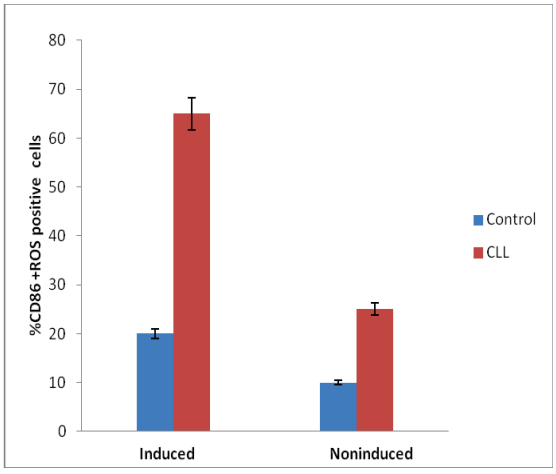


Fig. 2: CD86 expression (n=20)

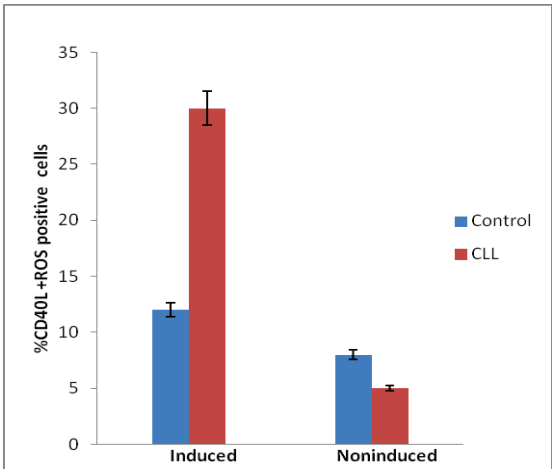


Fig. 3: CD40L expression (n=20)

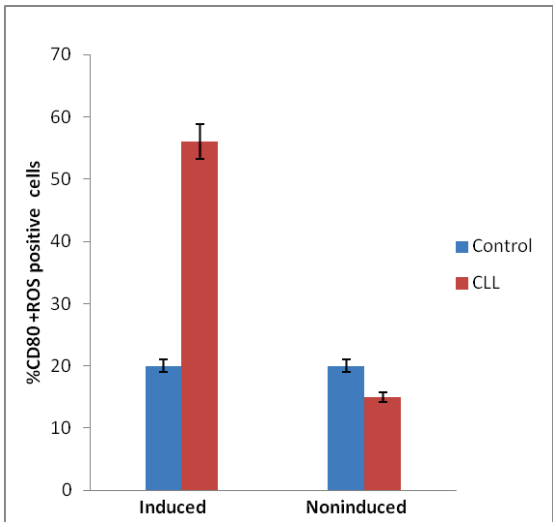


Fig. 4: CD80 expression (n=20)

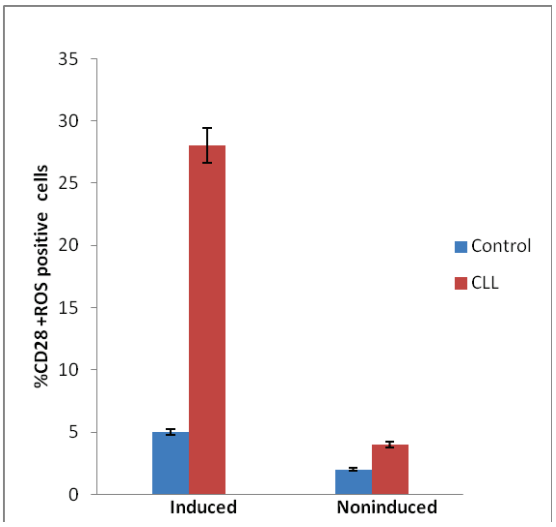


Fig. 5: CD28 expression (n=20)

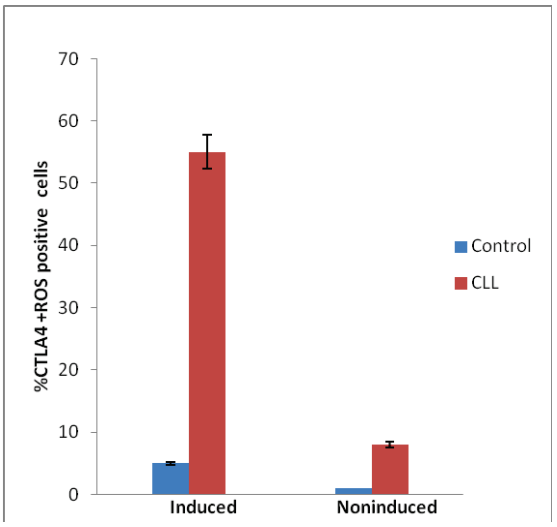


Fig. 6: CTLA expression was higher in induced cells as compared to induced cells.

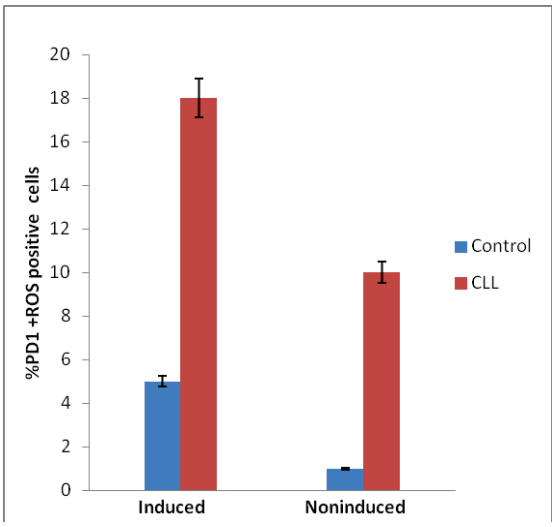


Fig. 7: PD1 expression was higher in induced cells as compared to non-induced cells.

Analysis of anti-oxidant enzymes:

B cells and T cells were purified from B-CLL patient PBMCs and cultured in condition media for 96 hours. Cells were centrifuged at 2000 x g for 10 minutes at 4°C and the cell pellet was homogenized in cold PBS. After further centrifugation, the supernatant was removed and stored until processed for enzyme assays. Assay was done using colorimetric based catalase and glutathione reductase assay. Catalase activity is shown in Fig. 8 and glutathione reductase in Fig. 9. Catalase and glutathione reductase activity was observed to be higher in CLL B cells as compared to normal B cells. They were also higher in CLL B cells as compared to CLL T cells. The higher activity of these anti-oxidant enzymes may be responsible for resistance to therapy and for longer survival of B cells in CLL.

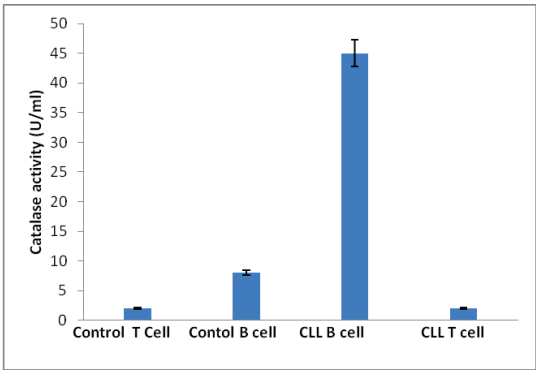


Fig. 8: Catalase activity assay (n=20)

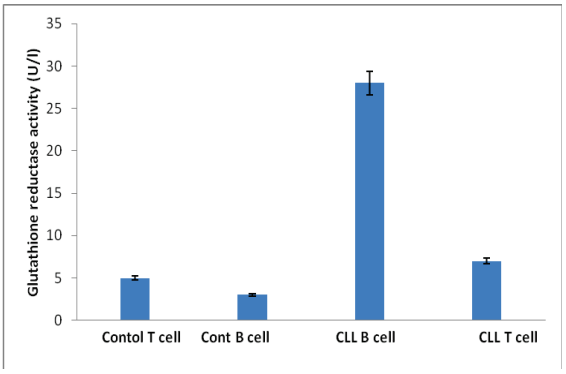


Fig. 9: Glutathione reductase assay (n=20)

BRAIN TUMORS

1. IMMUNOHISTOCHEMICAL EXPRESSION OF IDH1 IN GLIOMAS: A TISSUE MICROARRAY BASED APPROACH

Scientific staff	: Dr. Varuna Sipayya, Ms Ira Sharma, Dr. Avninder P Singh
In collaboration with	: Dr. KC Sharma, Department of Neurosurgery, Safdarjung hospital, New Delhi
Duration	: 2011-12

Aims, Objectives & Background

Gliomas arise from the glial cells and constitute about half of all primary intra-cranial tumors. Depending on the cell of origin, gliomas are mainly of three types: astrocytoma, oligodendroglioma and ependymoma. Malignant gliomas are the most frequent and lethal cancers originating in the CNS. These include astrocytoma, oligodendroglioma and ependymoma. The most frequent and biologically aggressive subtype is glioblastoma (GBM). Historically, GBMs have been categorized into two groups primary and secondary on the basis of their clinical presentation. Secondary GBM (sec-GBM) are defined as tumors that have clinical, radiologic or histopathologic evidence of malignant progression from a pre-existing lower grade tumor, whereas primary GBMs (prim-GBM) have no such history and present *de novo* as advanced cancers at the time of diagnosis. The histopathologic findings of primary and secondary GBMs are indistinguishable and their prognosis does not appear to be significantly different after adjustment for age. However, these variants of GBM differ significantly with respect to genetic alteration. EGFR amplification, MDM 2 amplification and PTEN mutations are typical of prim-GBM while TP53 mutations are more frequent in sec-GBM. Substantial research has been focused on identification of gene alterations in GBM that might help to stratify GBM patients depending upon prognosis and response to therapy.

The objective of this study is to compare immunohistochemical expression of IDH1 in low-grade and high-grade gliomas. With this objective, we analysed a series of 184

glioma cases in a tissue microarray based approach to assess the frequency of R132H point mutations in formalin fixed, paraffin embedded tissue samples.

Work done during the year

Mutations in the gene encoding isocitrate dehydrogenase (IDH1) have been reported in acute leukemias and gliomas. We screened a total of 195 gliomas for IDH1 mutations using a tissue microarray based approach and assessed the role of immunohistochemical expression of IDH1 protein in these tumors with respect to different histological types and to correlate IDH1 immunoexpression with p53 and EGFR expression.

A total of 195 gliomas (30 pilocytic astrocytoma (PA), 45 diffuse astrocytoma (DA), 75 glioblastoma multiforme (GBM), 25 oligodendroglioma (OLIG) and 20 ependymoma (EPEN). A TMA of core size 1.0 mm was constructed using a semi- automatic tissue arrayer. Immunohistochemical staining for IDH1, p53 and EGFR proteins was performed by LSAB method (Fig. 1). The immunoexpression of IDH1, p53 and EGFR will be correlated.

The summary of the results is given in Table 1. None of the PA or EPEN showed any immunostaining with IDH1protein. However, 17/40 (42.5%) DA showed positive labelling with IDH1 and 27/40 (67.5%) showed mutant p53. This IDH1 expression was positively correlated with p53 expression (p=0.018). OLIG also showed positive immunoexpression of IDH1 in 22.5% of cases but mutant p53 in only 41% cases (p=0.07). Among primary GBM, only 1/65 showed IDH1 immunopositivity whereas 6/7(85%) of sec-GBM showed expression of this protein. Prim-GBM showed p53 and EGFR positivity in 64.5% and 60% respectively. In the 6/7 sec-GBM that showed mutant IDH1 expression, 5/7 also showed mutant p53 but in GBM co-expression of IDH1 and EGFR was rarely seen. Also sec-GBM which showed IDH1 mutations more frequently in comparison to prim-GBM occurred in younger patients (mean age 42.5years) as compared to prim-GBM (mean age of 47.2 years). Follow-up and survival data of most of these patients was not available as ours is a tertiary referral hospital with more than half of patients coming from other towns and villages across India.

Table 1: Immunohistochemical expression of IDH1, p53, and EGFR in gliomas

Tumor	Mean age(yr)	IDH1+ N (%)	IDH1- N (%)	P53+ N (%)	P53- N (%)	EGFR+ N (%)	EGFR- N (%)
Pilocytic Astrocytoma	13.8	0/30 (0.0)	30/30 (100.0)	4/30 (13.3)	26/30 (86.6)	4/30 (13.3)	26/30 (86.6)
Diffuse Astrocytoma	39.2	17/40 (42.5)	23/40 (57.5)	27/40 (67.5)	13/40 (32.5)	12/40 (30.0)	28/40 (70.0)
Oligodendroglioma WHO grade 2	38.3	5/22 (22.7)	17/22 (77.2)	9/22 (40.9)	13/22 (59.0)	7/22 (31.8)	15/22 (68.1)
Primary GBM	47.2	1/65 (1.5)	64/65 (98.4)	42/65 (64.6)	23/65 (35.3)	39/65 (60.0)	26/65 (40.0)
Secondary GBM	42.5	6/7 (85.7)	1/7 (1.4)	5/7 (71.4)	2/7 (28.6)	0/7 (0.0)	7/7 (100.0)
Ependymoma WHO grade 2	21.9	0/20 (0.0)	20/20 (100.0)	10/20 (50.0)	10/20 (50.0)	4/20 (20.0)	16/20 (80.0)

Conclusion

Monoclonal antibody to IDH1 (R132) is a useful and less labor-intensive method to detect mutations in gliomas. IDH1 is a useful immunohistochemical marker to differentiate reactive gliosis from low-grade astrocytoma, has potential as an independent prognostic marker and also helps in distinguishing primary from secondary glioblastoma. Its sensitivity and specificity needs to be assessed by simultaneous sequencing and its validation on clinically annotated samples.

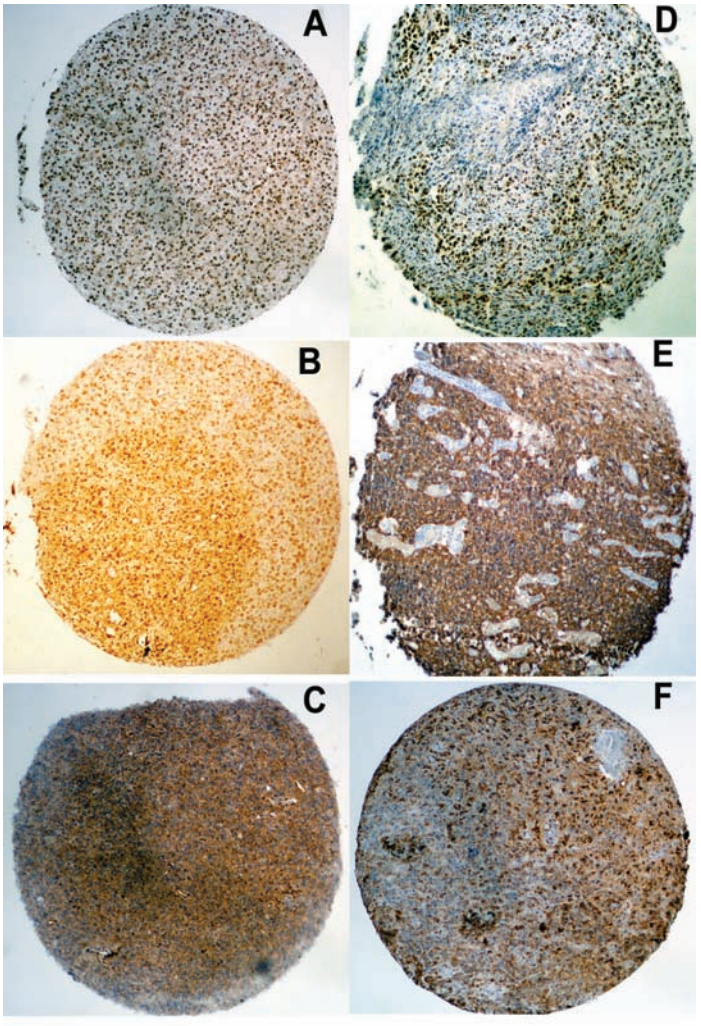


Fig. 1: Photomicrograph of tissue microarray cores showing isocitrate dehydrogenase1 (IDH1) expression in diffuse astrocytoma (DA), (A), glioblastoma multoforme (GBM). (B), Oligodendroglioma (C), p53 in GBM (D), EGFR in GBM and p53in DA (x100)


PATHOLOGY OF
INFECTIOUS
DISEASES

PATHOLOGY OF INFECTIOUS DISEASES

LEISHMANIASIS

1. DRUG RESISTANCE IN VISCERAL LEISHMANIASIS

Scientific staff	: Dr. Poonam Salotra, Dr. Ruchi Singh, Ms Arpita Kulshrestha, Ms. Vasundhra Bhandari, Mr. Deepak Kumar Deep
Collaborators	: Dr. NS Negi, Dr. V Ramesh
Duration	: 2010-14

A. Miltefosine (MIL) resistance in Visceral Leishmaniasis

Aims, Objectives & Background

Widespread resistance against Sodium Antimony Gluconate (SAG) has resulted in introduction of miltefosine (MIL) as the first line drug in parts of Bihar, however, long half-life treatment poses threat of development of resistance. Reports of relapses following MIL treatment have surfaced already. Hence, it is essential to monitor the treatment efficacy and understand the mechanism of resistance towards MIL for effective control of VL. During the period under study, we determined the *in vitro* susceptibility of VL and PKDL isolates following MIL treatment including relapse cases towards MIL. Further, we validated the results of microarray performed between MIL-resistant and sensitive parasites by real time PCR and based on the result propose the possible mechanisms of MIL resistance in *Leishmania donovani*.

As reported earlier, we have generated an experimentally resistant MIL strain upto 74μM (MIL-R designated as LdM30), in which the resistance induced at promastigote stage was also evident at amastigote stage. We have utilized MIL-R strain to explore the mechanism of experimental MIL resistance operative in *L. donovani.*

Work done during the year

1. Miltefosine susceptibility of clinical isolates

We measured *in vitro* susceptibility towards MIL in *L. donovani* isolated from MIL treated VL and PKDL cases, at pre- and post-treatment, using an amastigote-macrophage model. MIL susceptibility of post-treatment isolates from cured VL cases (n = 13, mean IC_{50}±SD = 2.43±1.44 µM), was comparable (p>0.05) whereas that from relapses (n = 3, mean IC_{50} = 4.72±1.99 µM) was significantly higher (p = 0.04) to that of the pre-treatment group (n = 6, mean IC_{50} = 1.86±0.75 µM) (Fig. 1).

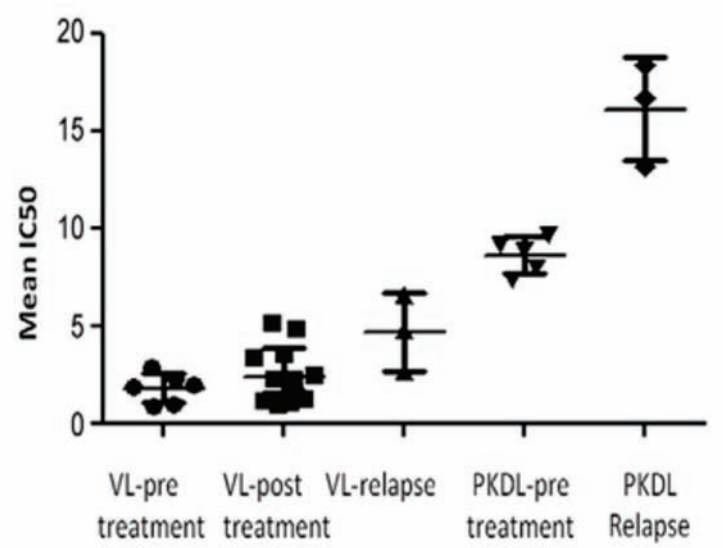


Fig.1: *In vitro* miltefosine susceptibility of parasite isolates from VL and PKDL cases before and after Mil treatment. Sensitivity of VL and PKDL isolates at intracellular amastigote stage were determined by infection in murine macrophage cell line J774A.1. Each individual value represents mean IC_{50}±SD of the results from two separate assays.

2. Analysis of experimental MIL resistance markers in field isolates

MIL transporter genes LdMT/LdRos3 were previously reported as potential resistance markers in strains in which MIL resistance was experimentally induced. The down-regulated expression of these transporters observed *in vitro* could, however, not be

verified in natural populations of parasites. mRNA expression level of LdMT and LdRos3 was analyzed in 19 VL and two PKDL isolates using real-time PCR in comparison to the reference strain *L. donovani* LdAG83. The expression was found comparable in all the groups including the relapse cases of VL and PKDL (Fig. 2). Expression pattern of LdMT/ LdRos3 genes, therefore, does not appear to be suitable marker for monitoring drug susceptibility in clinical *Leishmania* isolates. Thus, our data indicates that mechanism for MIL resistance in field may also involve genes other than LdMT and LdRos3.

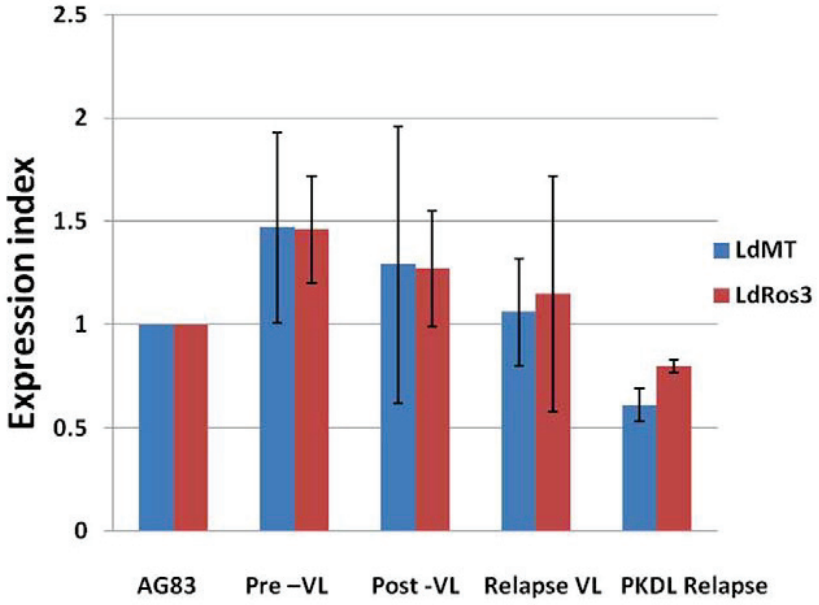


Fig. 2: Expression of LdMT and LdRos3 in clinical isolates of VL and PKDL. Real-time reverse-transcription PCR expression analysis of *L. donovani* MIL transporter genes (LdMT and LdRos3) was performed using GAPDH as internal control. Graph shows the expression index, defined as ratio of gene expression relative to that of strain LdAG83. Data represent the mean±SD of the results of three independent experiments.

3. mRNA expression analysis of LdMT and LdRoS3 in intermediate stages of LdM30 isolate

As shown above, we evaluated the mRNA expression of LdMT and LdRos3 in clinical isolates; however, we could not find any modulation of gene expression as in lab generated MIL R isolates. Further, we validated the expression of these markers in the intermediate stages of the LdM30 isolate (12, 18, 37, 49 and 74μM) to follow the expression pattern. We found that these markers remained unaltered in isolates with IC50 below 46μM and were downregulated (>1.5 fold) in isolates with higher IC50 (Figs. 3A-B).

mRNA expression of LdMT in the intermediate stages of Ld M30

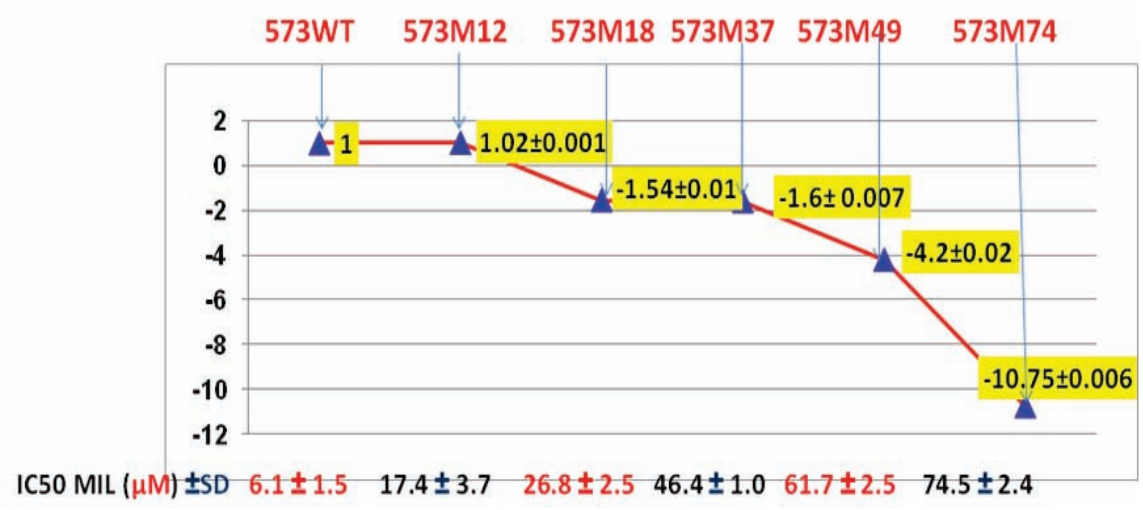


Fig.3A: RQ of gene at different stages of MIL adaptation of LdM30 is indicated with respect to wild type, LdWT.
Data represents mean±SD of 2 different experiments performed in triplicate.

mRNA expression of LdRos3 in the intermediate stages of LdM30

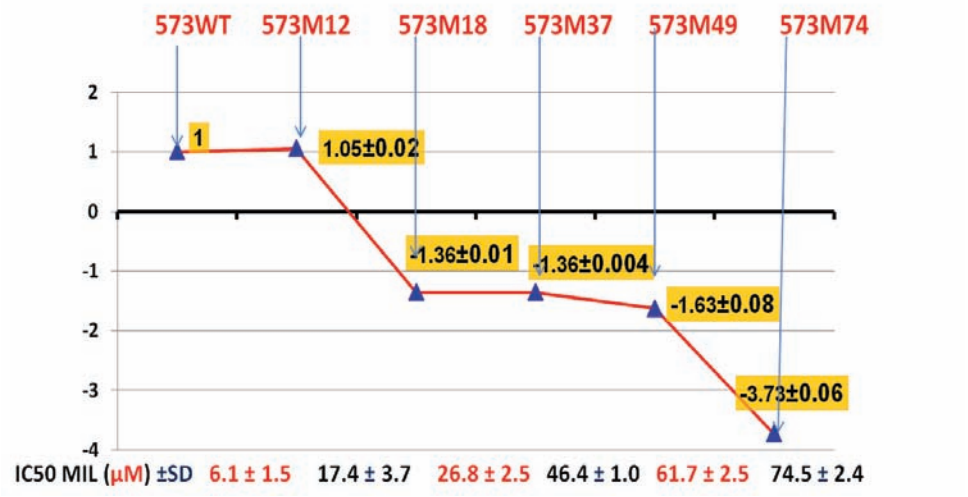


Fig.3B: RQ of gene at different stages of MIL adaptation of LdM30 is indicated with respect to wild type, LdWT.
Data represents mean±SD of 2 different experiments performed in triplicate.

4. Transcriptome profiling of MIL-R Vs MIL-S parasites

We employed genomic microarray analysis to compare the gene expression patterns of miltefosine resistant and sensitive *L. donovani*. Last year, we reported that 311 genes representing ~ 3.9% of the total *Leishmania* genome were differentially expressed in miltefosine resistant parasite. The expression of genes on all chromosomes, except chromosome 1, 2 and 3, were modulated more than 2 fold in *L. donovani* MIL-R, as suggested by the chromosome map of gene expression (Fig. 4). All modulated genes from chromosomes 5, 15, 23, 26 and 29 were upregulated (represented in red) while those on chromosome 6 were downregulated (represented in green). Gene expression modulations possibly occur in a grouped manner in the parasite genome.

In comparison to wild type parasite, MIL resistant parasite showed an upregulated DNA synthesis, transporter activities and downregulated protein metabolic process as determined by BLAST2GO, AmiGO and KEGG pathway analysis.

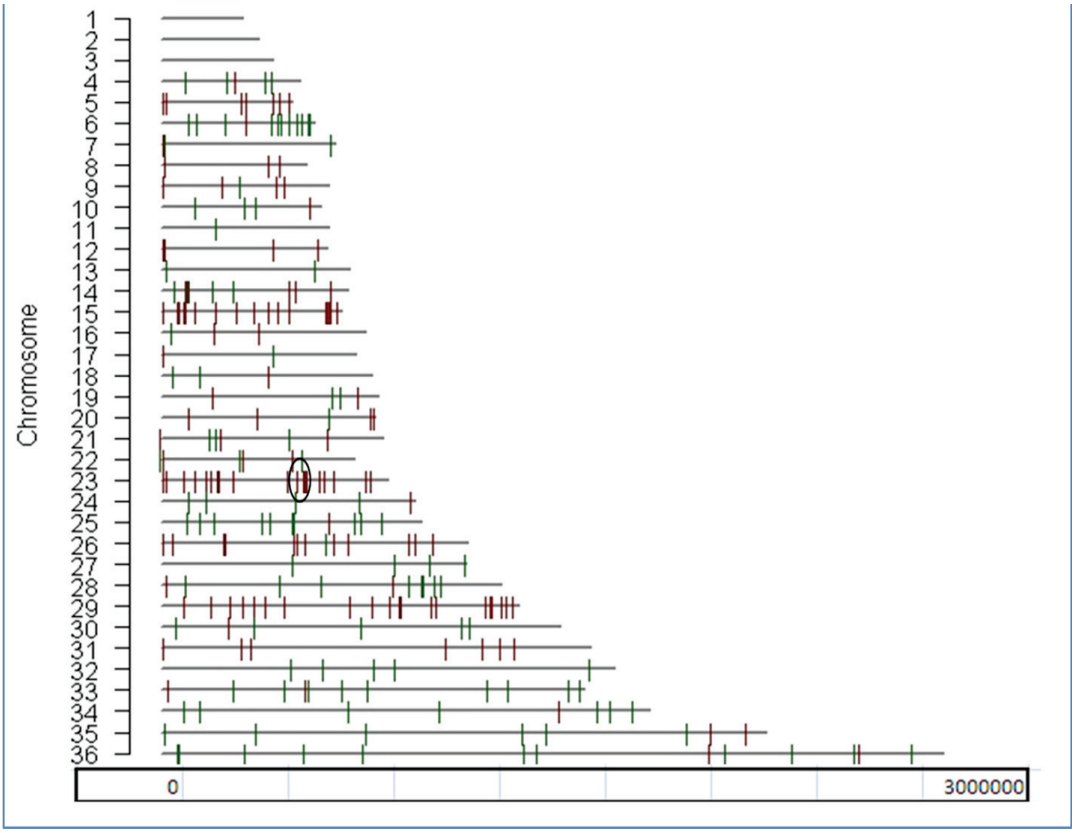


Fig. 4: Gene expression map of *L. donovani* Miltefosine resistant (MIL-R)/wild type (WT). DNA microarrays data were analyzed by custom R programs to illustrate the expression profile of *L. donovani* MIL-R/WT by extrapolating on a chromosome map of *Leishmania infantum*. Red lines indicate upregulated genes in MIL resistant parasite, whereas green lines indicate downregulated genes.

Current results suggest several probable mechanisms by which the parasite sustains miltefosine pressure including (i) increased ABC 1 mediated drug efflux (ii) reduced protein synthesis and degradation, (iii) altered energy utilization via increased lipid degradation and (iv) increased antioxidant defence mechanism via elevated trypanothione metabolism and reduced oxidative phosphorylation. Based on the data, we propose that the mechanism of MIL-R is multifactorial and involves various processes as depicted in Fig. 5. The study provides the first comprehensive insight into the underlying mechanism of miltefosine resistance in *L. donovani* that will help to design strategies to increase lifespan of this important oral anti-leishmanial drug.

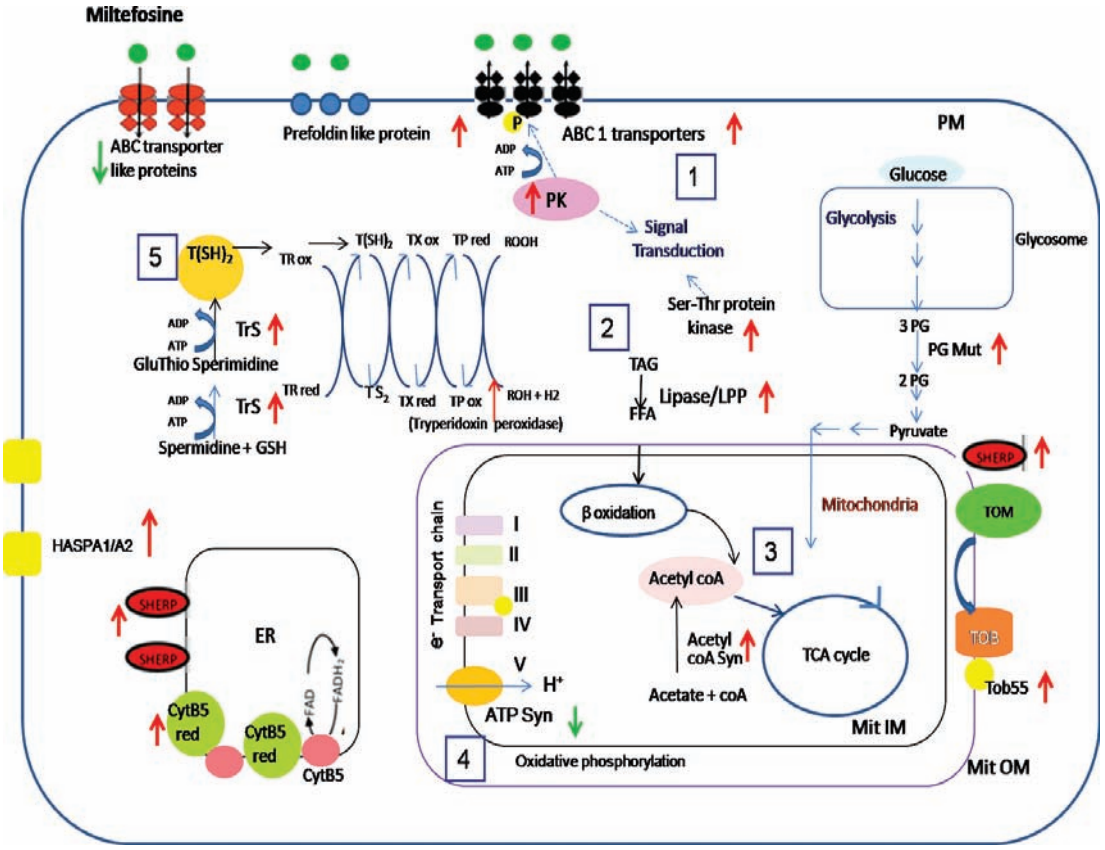


Fig. 5: Model depicting mechanism of Miltefosine resistance in *L. donovani*. Genes altered in MIL-R parasite are represented. Genes marked with red arrow represent the up-regulated genes and the ones marked with green arrow represent the down-regulated genes in case of MIL-R parasite. 1, 2, 3 and 4 represents the probable mechanisms of resistance in the MIL-R isolate. **(1)** ABC 1 transporters (ABCA1 and A7) up regulated in MIL-R that lead to efflux of MIL out of the cell. Protein kinase (PK), known to phosphorylate the ABC1 transporters; stabilize the expression of these proteins on plasma membrane, possibly contributing to MIL-R. **(2)** Lipases up regulated in MIL-R are involved in fatty acid metabolism and free fatty acids (FFA) from lipid degradation could be destined to beta oxidation for energy generation as an alternate energy source **(3)** Upregulation of AcetylcoA synthetase (involved in TCA cycle) in MIL-R **(4)** Reduced oxidative phosphorylation (due to down regulated expression of ATPase beta subunit, vacuolar ATP synthase subunit B and ATP synthase) contributes to reduced reactive oxygen species generation and prevents oxidative damage in MIL-R. **(5)** Upregulation of Trypanothione [T(SH)2] biosynthesis by trypanothione synthetase (TrS) in MIL-R enhances the anti-oxidant metabolism of *Leishmania* parasites thereby contributing to MIL resistance. Up regulation of tryparedoxin peroxidase [TP (ox)], responsible for hydroperoxide detoxification further aids in antioxidant defense in MIL-R.

GSH-Glutathione, [T(SH)2]-Trypanothione, (TrS)-trypanothione synthetase, TP(ox)-tryparedoxin peroxidase, CytB5-CytochromeB5, CytB5 red-CytochromeB5 reductase, ER- Endoplasmic reticulum, SHERP- Small Hydrophilic Endoplasmic Reticulum Associated Protein, PK-Protein kinsae, ATP syn- ATP synthase, PG Mut- Phospho glycerate mutase, TOM- Translocators of outer membrane, Tob- Topogenesis of mitochondrial outer membrane β-barrel proteins, Mit OM- Mitochondrial outer membrane, Mit IM- Mitochondrial inner membrane, HASPA- Hydrophilic acylated surface protein A, LPP- Lipase precursor like proteins, FFA-free Fatty acid, TAG-Tri acyl glycerol.

Validation of microarray result

Out of a total of 311 genes found modulated in experimental LdM30 isolate, 10 genes were selected for expression analysis by Q-PCR. These included small hydrophobic endoplasmic reticular protein (SHERP), hypothetical protein, milton, cAMP phosphatase, cytochromeB5 reductase, mitochondrial transmembrane protein, lipase precursor like protein, lipase putative, ATPase, and metallopeptidases which are implicated in different pathways like cellular transport, lipid metabolism and oxidative phosphorylation. The expression of all the genes in three different MIL-R strains showed comparable expression level to that of the microarray results (Fig. 6). These genes were further validated by real time PCR in other two lab generated MIL-R isolates, showed similar expression level to that of the LdM30.

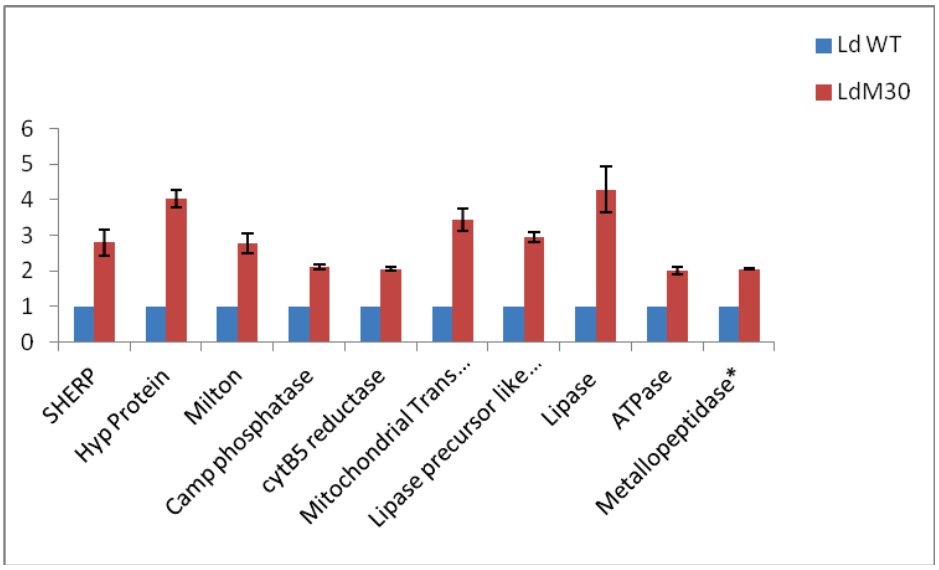


Fig. 6: Expression analysis of modulated genes was performed using GAPDH as internal control by Real Time PCR. Graph shows the expression index, defined as ratio of gene expression relative to that of LdWT. Data represents the mean±SD of the results of three independent experiments.

Future work: Overexpression of the selected gene (s) in *Leishmania* and monitoring of the parasite phenotype for the altered sensitivity towards miltefosine. Single nucleotide polymorphism will be analyzed in genes over-expressed in MIL-R parasites.

B. Paromomycin (PMM) resistance in Visceral Leishmaniasis

The phase IV trials reports for Paromomycin (PMM) confirm its safety and efficacy against VL. Hence, it is essential to understand the mechanism of resistance towards PMM for effective control of VL. During the period under study, we determined the in vitro susceptibility of VL and PKDL isolates following MIL treatment including relapse cases towards PMM. We have utilized PMM-R strain to explore the mechanism of experimental PMM resistance operative in *Leishmania donovani.*

1. Drug susceptibility of clinical isolates towards PMM:

Intrinsic sensitivity towards PMM was evaluated in a set of clinical isolates belonging to hyperendemic region of Bihar in order to obtain baseline data of susceptibility prior to its future use in therapy. The PMM susceptibility of 22 VL isolates, of which 6 were at pre-treatment stage and 16 were exposed to MIL treatment, was evaluated at intracellular amastigote stage. The IC_{50} ranged from 3.41±0.29 to 10.70±1.12μM with mean IC_{50}= 7.05±2.24μM. Furthermore, the PMM sensitivity was similar ($p > 0.05$) in parasites non-exposed (mean IC_{50} = 7.73±2.25μM) or exposed to MIL treatment (mean IC_{50} =6.79±2.25μM) (Fig. 7).

The inherent PMM susceptibility of 8 PKDL isolates ranged from 4.92±0.34 to 8.62±1.82μM. The PMM IC_{50} of PKDL isolates was similar ($p>0.05$) in parasites non-exposed (n=5, mean IC_{50} =6.12±1.40μM) or exposed to MIL (n=3, mean IC_{50}= 6.29±2.02μM) (Fig. 7). The drug susceptibility of VL and PKDL isolates to PMM was similar ($p> 0.05$).

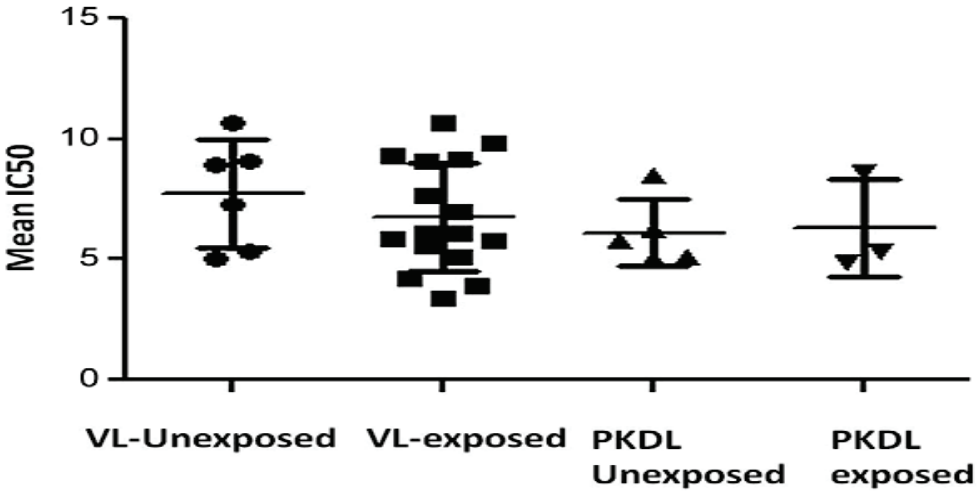


Fig. 7: PMM susceptibility profile of VL and PKDL isolates exposed or non-exposed to MIL treatment. Susceptibility of VL and PKDL isolates at intracellular amastigote stage was determined by infection in murine macrophage cell line J774A.1. Each individual value represents mean IC50±SD of the results from two separate assays.

Mechanism of PMM resistance

In the last year report, we had described the generation of an experimentally resistant PMM strain up to 97μM (PMM-R) in which the resistance induced at promastigote stage was also evident at amastigote stage. The laboratory generated PMM resistant line (PMM-R) of *Leishmania* was utilized to explore the mechanism of PMM resistance.

a) Increased membrane fluidity of the parasite: Diphenylhexatriene (DPH) has been used as fluorescence probe to measure fluidity of the membrane. The fluorescence anisotropy value of DPH was used as a measure of membrane fluidity; anisotropy being inversely proportional to membrane fluidity. Anisotropy was 2.5 times lower in PMM-R parasites compared to wild type (WT), indicating modifications towards higher fluidity in the parasite membrane due to PMM resistance (Fig. 8).

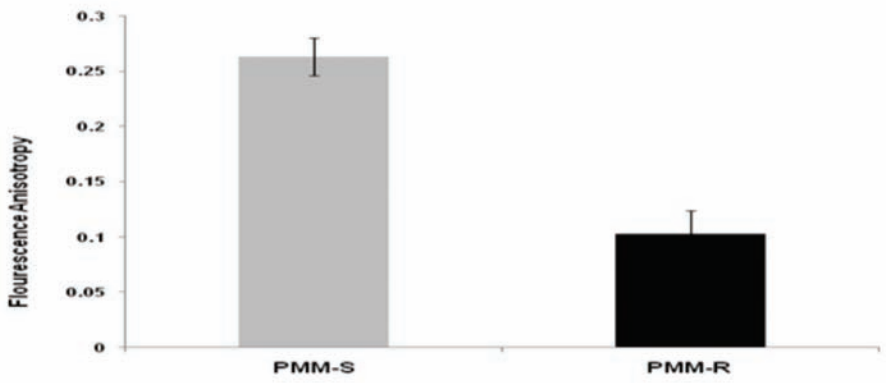


Fig. 8: Analysis of anisotropy of PMM resistant and sensitive *L. donovani*. Fluorescence anisotropy as a measure of membrane fluidity of PMM-S and PMM-R parasites was estimated using DPH. Data represent the mean ± SD of the results of three independent experiments.

b) Decreased drug accumulation: We further analyzed the PMM accumulation in the parasite with the help of LC-MS. The drug uptake of PMM-R isolate was decreased with an increase in the incubation time from 0-90 mins as compared to WT. There was approx. 3 fold decrease in drug uptake of PMM-R strain as compared to WT (Fig. 9).

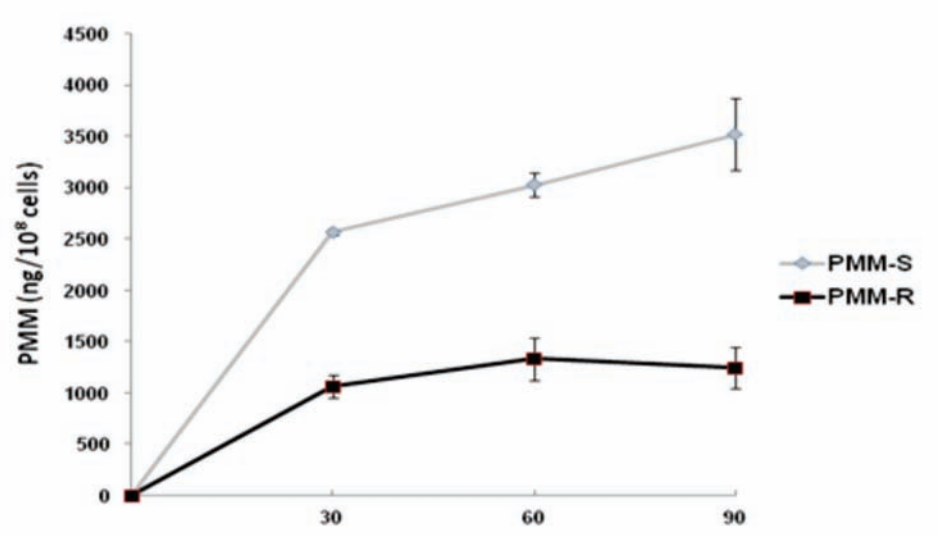


Fig. 9: Drug accumulation in *L. donovani* (PMM-S) and PMM-R promastigotes after incubation with 100μM of PMM. Values are means of two independent experiments and standard deviations are represented by error bars.

c) rRNA sequence analysis: PMM resistance have been associated with mutations in rRNA gene in bacteria, therefore we performed the sequence analysis of its gene homologue in *Leishmania* (*LinJ.27.rRNA6*) by direct sequencing of the amplicons resulting from the PCR with PMM-S and PMM-R parasite DNA. There was 100% similarity between the isolates with no mutations identified.

Future work: To determine the innate susceptibility of PMM-R isolate against host microbicidal responses including nitrosative, oxidative and complement mediated stresses. Comparative transcriptomic profiling of PMM resistant *L. donovani* isolate using genomic microarray technology to identify genes showing altered expression in PMM-R isolate followed by functional characterization of the selected gene (s).

2. STUDIES ON IMMUNOLOGICAL RESPONSES TO RECOMBINANT PSA (PARASITE SURFACE ANTIGEN)- A POTENTIAL VACCINE CANDIDATE

Scientific staff	**:** Dr. Poonam Salotra, Mr. Himanshu Kaushal, Mr. Deepak Kumar Deep
In collaboration with	**:** Dr. V Ramesh, Dr. NS Negi, SJ hospital, New Delhi
Duration	**:** 2010-12

Aims, Objectives & Background

Different *Leishmania* antigen preparations have been studied as vaccine candidates with different degrees of results, mainly in mice models. Recently, an effective canine vaccine based upon LiESAp, a crude excreted- secreted antigen obtained from promastigote culture supernatant of *Leishmania infantum*, formulated with muramyl dipeptide (MDP) was developed in France. Native soluble *L. infantum* Parasite Surface Antigen (nsLiPSA) has been identified as the active constituent of LiESAp. The PSA is a naturally excreted secreted protein belonging to the family of Promastigote Surface Antigens and has been produced in a non-pathogenic *L. tarentolae* recombinant expression system at our collaborator's lab (Institut pour la Recherche et le Développement, Montpellier, France).

As the nsLiPSA is present in all *Leishmania* species and showed 100% homology with PSA of *L. donovani*, therefore, we investigated immune responses to PSA in comparison with total soluble Leishmania antigen (TSLA), in order to evaluate its potential as immunoprophyactic antigen for human leishmaniasis.

Work done during the year

Cellular immune responses in peripheral lymphocytes from VL, PKDL, healed VL and naïve individuals: Generalized cellular mediated immunity was analyzed in terms of lymphoproliferative responses to PSA and TSLA with phytohemagglutinin (PHA) as positive control. VL cases showed hardly any proliferation (SI Mean±SD, 1.329±0.539), that was comparable to the naïve group (SI Mean±SD, 1.190±0.28). Healed VL (SI Mean±SD, 5.99±1.987) and PKDL (SI Mean±SD, 3.353±3.972) group showed significantly high stimulation compared to naive group (Fig. 10). PSA induced poor lymphproliferation in VL and PKDL and marginally significant (P= 0.047) in HVL group (Fig. 10).

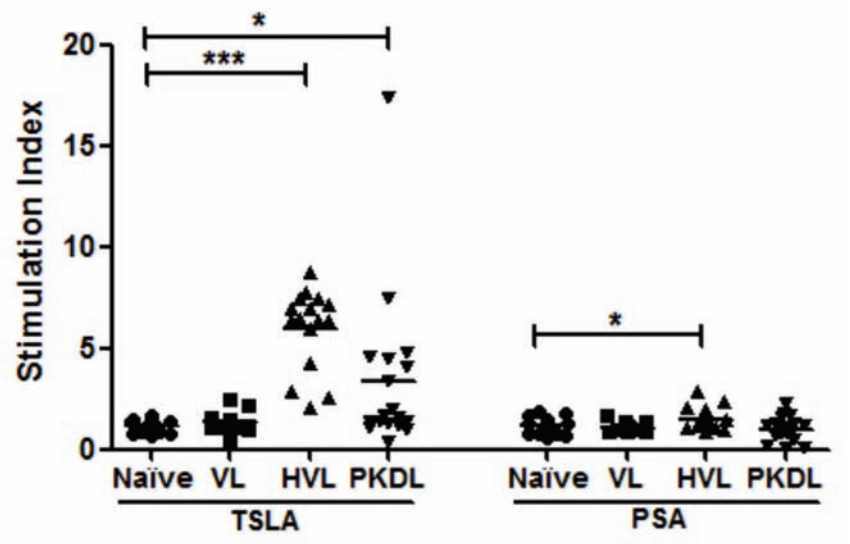
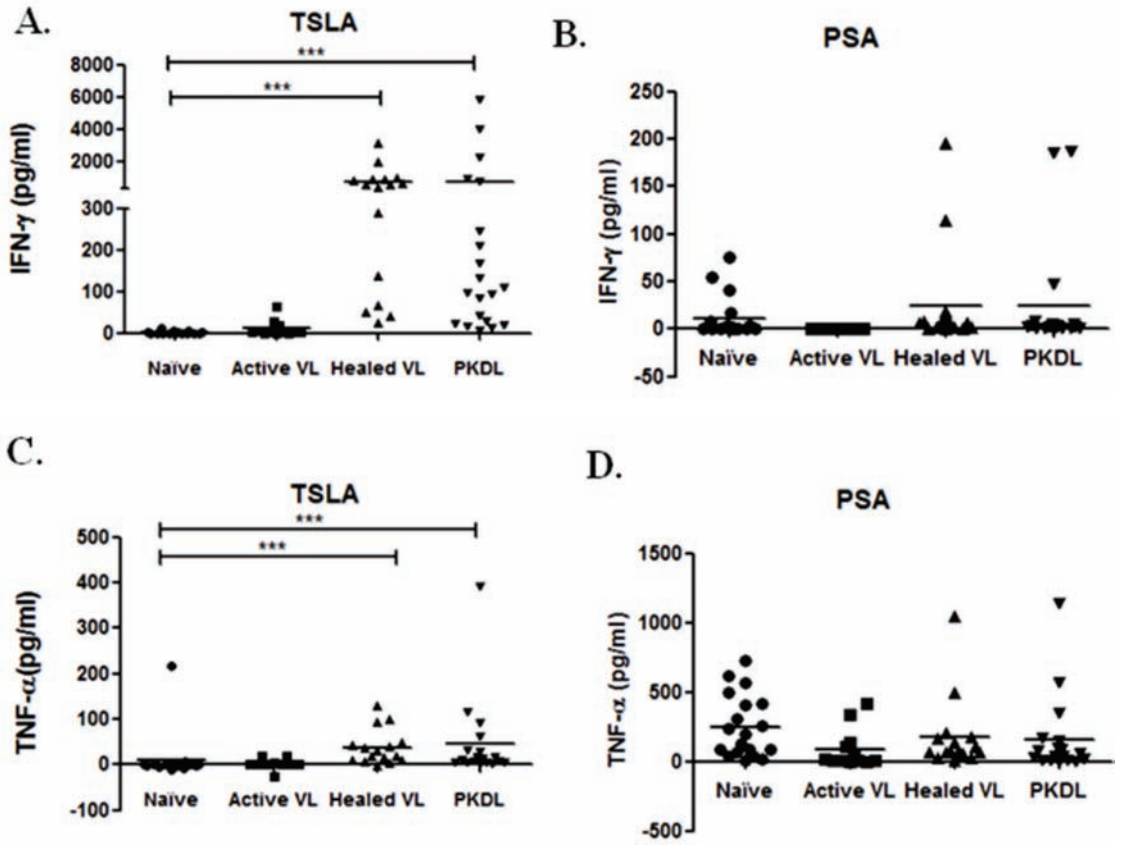


Fig.10: Proliferative responses of lymphocytes to TSLA and PSA. Lymphocytes from patients with PKDL (n=18), VL (n=12), healed VL (n=16) and from healthy group (n=19) were incubated with PSA (10µg/ml), TSLA (10µg/ml) or PHA (10µg/ml) for 5 days and lymphoproliferation was measured by BrdU incorporation for the last 12-14 hrs using Biotrak cell proliferation ELISA system. Data were analyzed with respect to naïve group by the nonparametric Mann Whitney test. $P<0.05$ is considered statistically significant. *$P<$ 0.05, **$P<0.01$, *** $P<0.001$

Cytokine analysis in response to TSLA or PSA stimulation

PBMCs from patients with active VL, PKDL and individuals from cured VL and healthy groups were screened for their Th1/Th2 cytokines responses to TSLA and PSA. In PKDL and healed VL cases, the CMI responses (as judged from the levels of Th1 cytokines, IFN-γ and TNF-α) was found significantly higher (P<0.001) than healthy control subjects upon TSLA stimulation. PKDL group showed significantly high IFN-γ production (pg/ml) (512.47±1053.26) against TSLA compared to naïve group (1.80±2.5). Healed VL group also showed high IFN-γ with Mean±SD, 738±824 pg/ml whereas VL group showed low level of stimulation. Significantly high TNF-α production was observed to TSLA stimulation both in PKDL (P<0.01) and in healed VL (P<0.001) groups with Mean±SD, pg/ml 44.69±93.81 and 37.50±38.61 respectively compared to naïve group (10.915±49) whereas VL group showed low value with Mean±SD, 1.27±11.33. For IL-10 cytokine, the measured values for PKDL, healed VL and VL groups were low and comparable to naïve group. PSA failed to stimulate IFN-γ, TNF-α or IL-10 in any of the study group (Fig. 11).



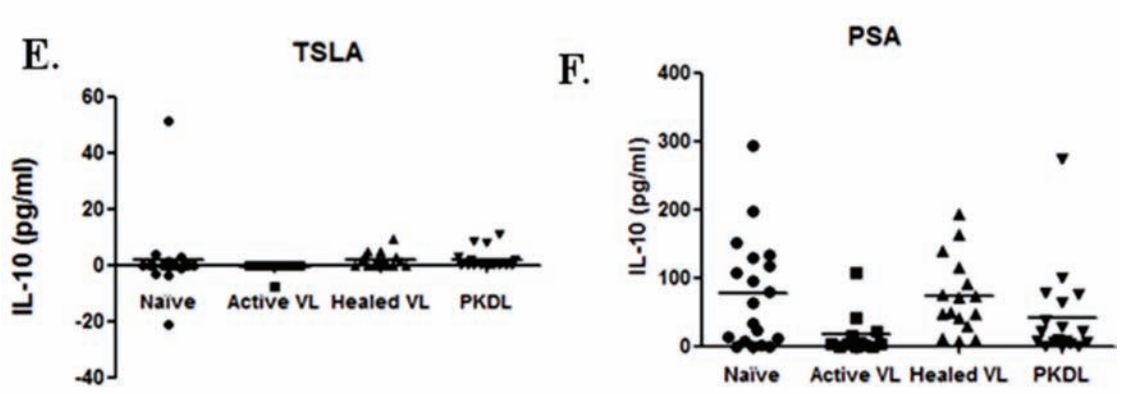


Fig. 11: *Leishmania*-specific cell mediated immune response in VL, PKDL, healed VL and healthy controls. PBMCs were isolated from the PKDL (n=18), VL (n=12), healed VL (n=16) and from healthy group (n=19) and were incubated with PSA (10µg/ml), TSLA (10µg/ml) and PHA (10µg/ml) for 5 days. Cytokines were measured in culture supernatant by using Cytometric Bead Array (CBA) flex (*BD Biosciences*).

Humoral response to TSLA and PSA

Serum samples from patients with active VL (n=25), PKDL (n=25), healed VL (n=10) and healthy controls (n=25) were tested for presence of anti-TSLA and anti-PSA IgG and IgM. Total anti-TSLA IgG were significantly ($P< 0.001$) elevated in active VL (Mean±SD OD, 1.575±0.64), PKDL (Mean±SD OD, 1.259±0.53) and healed VL Mean±SD OD, 0.663±0.71) compared to naïve (Mean±SD OD, 0.078±0.06) whereas anti-PSA total IgG in active VL, healed VL and PKDL were comparable to naïve group. Taking the cut-off value as 0.258 (Mean+3SD of naïve group), 25/25 of VL group, 25/25 of PKDL, 6/10 of HVL group and 1/25 of naïve group were positive for IgG against TSLA, confirming the diagnostic value of TSLA for VL and PKDL. IgM levels to anti-TSLA were found significantly ($P< 0.001$) high in active VL group (Mean±SD OD, 1.199±0.812) compared to control (Mean±SD OD, 0.319±0.239). Similar trend were observed for anti-PSA IgM. Significantly ($P< 0.01$) high IgM levels were found in active VL (Mean±SD OD, 1.099±0.824) compared to naïve (Mean±SD OD, 0.527±0.524) (Fig. 12).

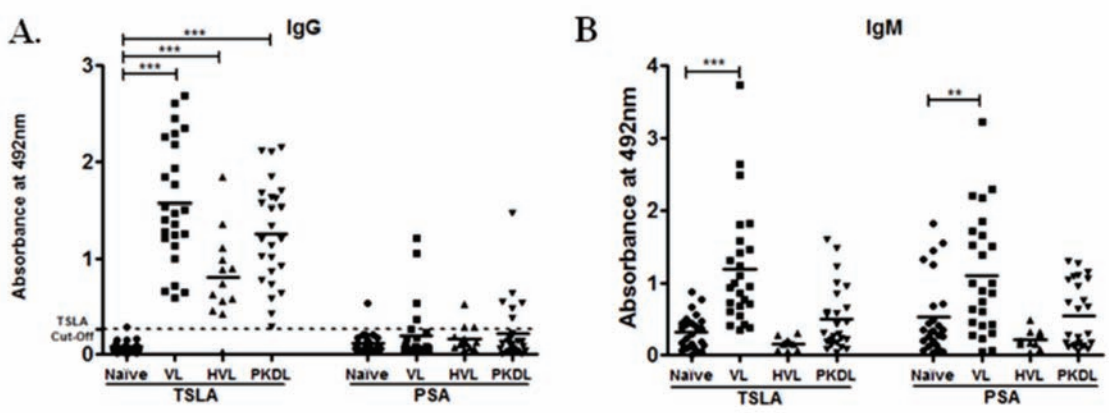


Fig. 12: Comparison of antileishmanial antibodies toTSLA and PSA of IgG and IgM in sera (1:100) of active VL healed VL, PKDL and healthy controls. The Mean±SD OD levels were determined by ELISA using horseradish peroxidase (HRP) - conjugated anti-human IgG and IgM and Ortho-phenylene diamine as substrate (SIGMA FAST OPD - ready-to-use substrate). The bars represent the means for the different groups.

3. IMMUNE RESPONSES AGAINST CENTRIN KNOCK OUT LIVE ATTENUATED LEISHMANIA PARASITES IN THE HUMAN CELLS

Scientific staff	:	Dr. Poonam Salotra, Mr. Kumar Avishek
In Collaboration with	:	Dr. Angamuthu Selvapandiyan, Dr. Hira Nakhasi, Dr. NS Negi
Duration	:	2011-14

Aims, Objectives & Background:

To date, no vaccine is available for leishmaniasis; hence, development of a safe and effective vaccine is required. Live attenuated parasite developed through gene knock out approach could be an ideal vaccine candidate as it possesses genetically defined, irreversible gene defect. Centrin1 is acalcium binding basal body associated protein involved in cell division in protozoan parasites like *Leishmania.* Deletion of Centrin1 gene

in *L. donovani* (*LdCen1*$^{-/-}$) results in attenuation of mammalian infecting, amastigote form with no effect on growth of promastigotes form of the parasite. Mice immunized with *LdCen1*$^{-/-}$ showed protective immune response against virulent challenge and long lasting immunity. This study aims to evaluate immune responses generated by Centrin knock out (cKO) Leishmania parasite in comparison to the wild type in human cells.

Work done during the year

In the year under report, we have measured IFN-gamma and IL-10 cytokine production in the PBMCs from a small number of Healed VL and healthy individuals after infection with wild type (WT) and cKO. Further macrophage infection protocol was also standardized using human PBMC, in order to study infectivity and cytokines stimulation in macrophages in response to WT *L. donovani* and cKO parasites.

1. Evaluation of IFN-gamma and IL-10 produced in human PBMCs in response to wild type and Centrin KO Leishmania parasite

Cytokines were estimated in the supernatant of PBMC after 5 days of infection with parasites in Healed VL (HVL, n=3) and Healthy samples (n=3). PBMC from healthy individuals showed stimulation of IFN-γ in comparison to control uninfected cells (1.09±0.72pg/ml) upon infection with WT (14.62±5.42 pg/ml) as well as cKO parasite (5.58±1.41pg/ml). PBMC from HVL blood, showed much higher stimulation with both WT (129.69±79.63pg/ml) and cKO(100.90±48.39pg/ml) parasites in comparison with control uninfected cells (14.38±1.55 pg/ml) (Fig. 13). The positive control PHA showed high IFN-γ concentration in both healthy (1049.15±208.57pg/ml) and HVL samples (950±193.70pg/ml). Studies are being extended to large number of samples in each group to obtain statistical significance.

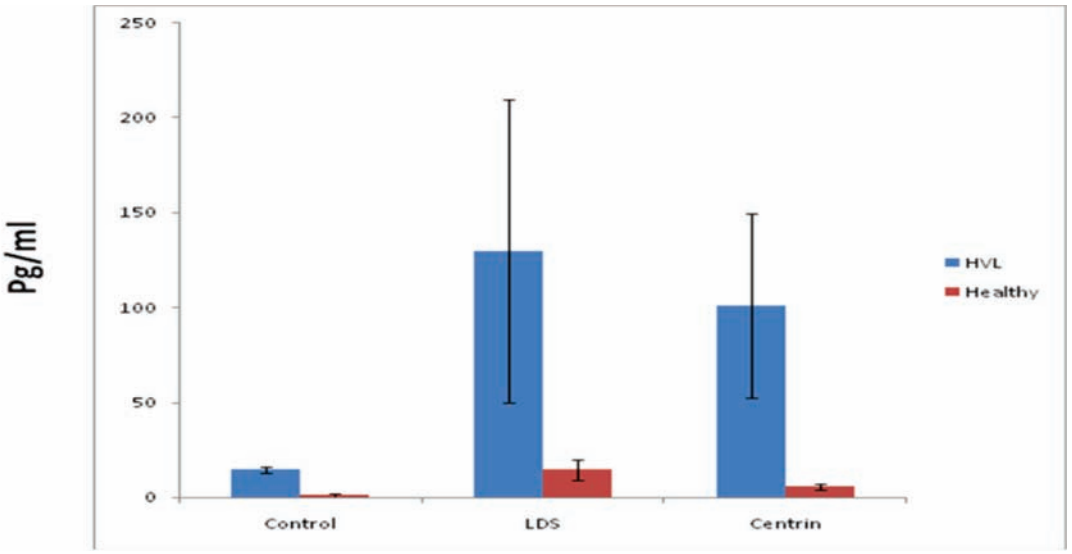


Fig. 13: Levels of interferon (IFN) -γ in HVL and healthy samples determined by sandwich ELISA. Values given in pg/mL as mean±SEM.

In case of IL-10, there was only marginal stimulation with WT (LDS) and cKO parasite in both healthy (WT=2.38±4.49pg/ml, cKO=8.74±5.28pg/ml) and HVL (WT=9.38±3.60pg/ml, cKO=7.08±1.91pg/ml) samples although PHA showed good stimulation (Fig. 14).

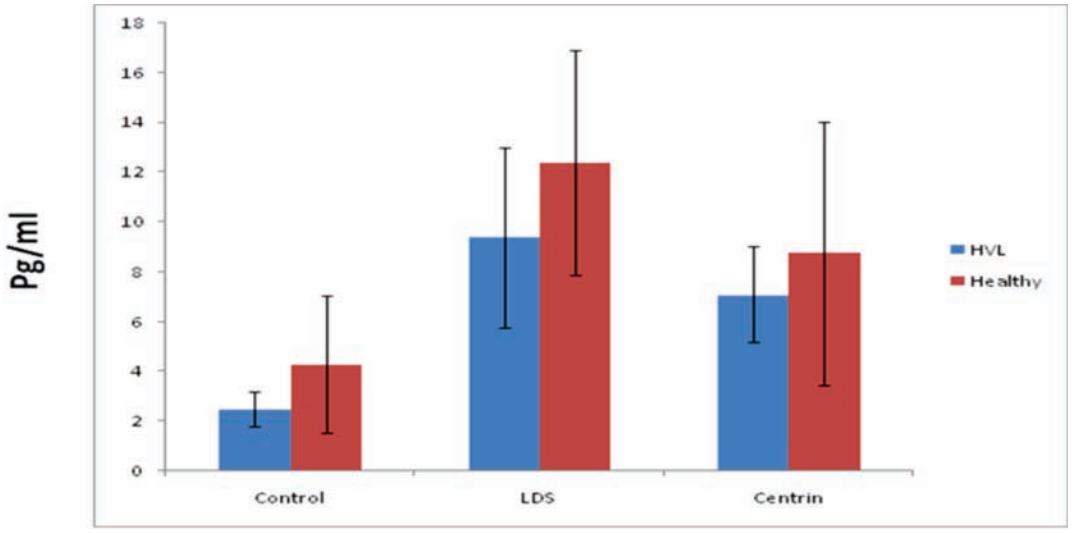


Fig. 14: Level of IL-10 in HVL and healthy samples determined by ELISA. Values are given as mean±SEM.

Infectivity of wild type and Centrin KO Leishmania parasite in PBMC differentiated host macrophages

Human PBMC differentiated macrophages were infected with both WT and cKO *L. donovani* parasites. Adequate infection was observed using macrophage to parasite ratio 1:10 for both (Fig. 15). Percent Infectivity of macrophages was found 70-80% in both WT and cKO parasite.

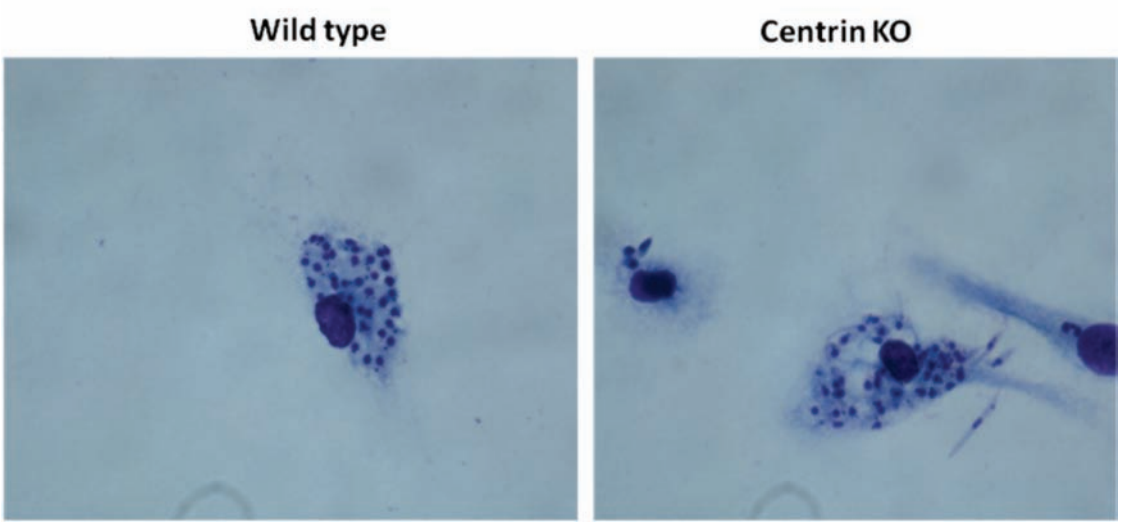


Fig. 15: Infection of human macrophages with wild type (left) and Centrin KO parasites (right). Human monocyte-derived macrophages were infected with parasites and were fixed and stained with Diff-Quik staining

Future Plan

Cytokines study in parasite infected PBMC supernatant will be extended to large number of samples of healthy, HVL and kala-azar categories in order to obtain statistical significance. Further, stimulation of other cytokines (IL-2, 4, 6, 10, 12, 17, IFN-γ, TNF-α & TGF-β) will be also evaluated. Cytokine production in macrophages infected with cKO will be also measured.

4. DIAGNOSIS OF VL AND PKDL USING LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP)

Scientific staff	:	Dr. Poonam Salotra, Mr. Sandeep Verma, Mr. Kumar Avishek, Ms Vanila Sharma
Duration	:	2011-12

Aims, Objectives & Background

VL and PKDL diagnostic methods based on parasite detection (stained smears, culture or histopathology) are invasive and have poor sensitivity, while immunological methods (Direct Agglutination Test, enzyme-linked immunosorbent assay etc.) are not conclusive for PKDL because of persistence of anti-leishmanial antibodies after VL, and are not reliable in immune-compromised patients. Previously we reported Real-time PCR (Q-PCR) assay as a sensitive and specific diagnostic tool for VL and PKDL diagnosis. We showed the utility of minimally invasive slit aspirate specimen for serological (rk39 strip test) screening and confirmation by molecular test (Q-PCR) for PKDL diagnosis. In the year under report, we have checked the utility of LAMP assay as a diagnostic tool for VL and PKDL. The method consists of incubating a mixture of target DNA with four different primers and *Bst* DNA polymerase for 1 hour at 60–65°C, using basic equipment such as a heat block or water bath. Shorter reaction time with visual judgment of positivity without requiring sophisticated equipment makes LAMP an attractive diagnostic method.

Work done during the year

Sensitivity and specificity of LAMP assay

The sensitivity of the LAMP assay was determined by serially diluted known amount of parasite DNA (10 ng – 1 fg). The reaction allowed a detection limit of 1 fg DNA, which is equivalent to DNA from less than 1 parasite. The LAMP assay was positive for *L. donovani* DNA and negative for other *Leishmania* spp. (*L. infantum, L. tropica* and *L. major*) using 10 ng DNA of each species. These results showed that the LAMP assay is specific for *L. donovani*. The LAMP amplified product was analyzed by electrophoresis

in 1.5% agarose gels stained with ethidium bromide (Fig. 16). However, this method of detection is tedious and time consuming needing equipment for gel electrophoresis. Therefore LAMP products were analyzed by addition of 1 µl of 1/10 dilution of SYBR Green I (*Molecular Probes Inc.*), which is a direct method of amplification detection by naked eye. All negatives remained orange while positive samples produced a green colour almost immediately (Fig. 17).

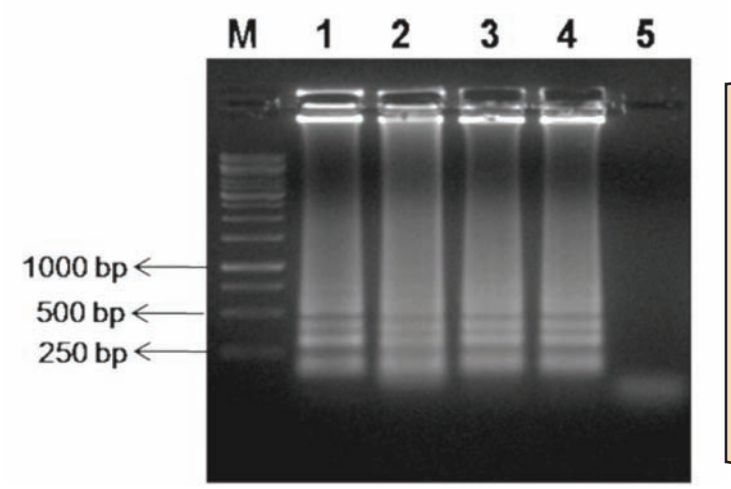


Fig.16: Analysis of DNA amplification of LAMP product by electrophoresis in 1.5% agarose gels stained with ethidium bromide. Lane M 1 kb ladder; lanes 1, 2, 3, and 4, 1 ng, 100 pg, 10 pg, 1 pg, of *L. donovani* DNA respectively; lane 5, water (negative control).

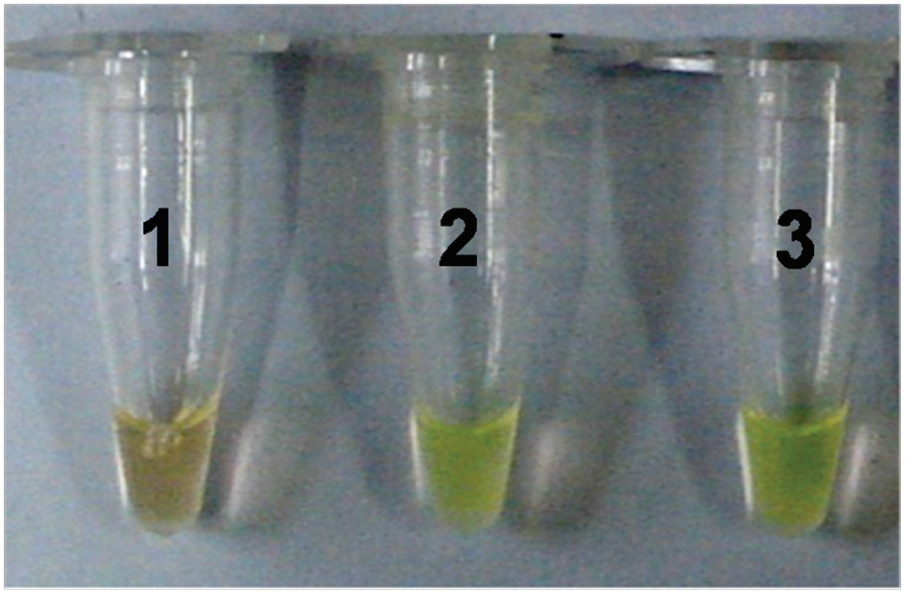


Fig. 17: Visual appearance of LAMP reactions after the addition of 1µl of 1/10 dilution of SYBR Green I. Negatives remained orange (tube 1, no template control) while positive samples produced a green color almost immediately (tubes 2 and 3 containing 1 pg and 1 ng of *Leishmania donovani* DNA respectively).

LAMP assay in clinical samples of VL and PKDL

LAMP reaction was performed using *Leishmania donovani* kDNA based primer sets. DNA isolated from blood and bone marrow aspirates of VL patients, tissue biopsy of PKDL patients and from control samples were subjected to LAMP assay.

The results of LAMP assay for VL and PKDL diagnosis are shown in Table 1. The LAMP assay was positive in 53 of 55 VL blood samples, giving sensitivity of 96.4% (95% Confidence interval, 87.7 – 99.0%). The LAMP assay was positive in all VL BMA samples (n=15), giving sensitivity of 100% (95% Confidence interval, 79.6 – 100%).The LAMP assay was positive in 60 out of 62 PKDL tissue biopsy samples, giving sensitivity of 96.8% (95% Confidence interval, 88.9 – 99.1%). The LAMP assay was negative for 67 out of 68 control samples (blood, n=44; tissue biopsy, n=24), giving specificity of 98.5% (95% Confidence interval, 92.1 – 99.7%) for *L. donovani* DNA detection.

Table 1: Sensitivity and specificity of the LAMP assay for diagnosis of VL (using blood and BMA samples) and PKDL (using tissue biopsy)

Sample	Cases tested	Cases positive	Sensitivity/Specificity (95% CI)
VL (Blood)	55	53	96.4% (87.7-99%)
VL (BMA)	15	15	100% (79.6-100%)
PKDL (Tissue Biopsy)	62	60	96.8% (88.9-99.1%)
Controls	68	1	98.5% (92.1- 99.7%)

CHLAMYDIASIS

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

Scientific Staff	:	Dr. Sangita Rastogi, Mrs. Priya Prasad
In collaboration with	:	Dr. Banashree Das, SJ hospital, New Delhi
Duration	:	2008-12

Aims, Objectives & Background

Oxidative attack by reactive oxygen species as a result of disturbed oxidant-antioxidant system balance is recognized in the pathogenesis of placental deficiency syndromes such as preeclampsia and fetal growth restriction. It is speculated that early pregnancy failure might also be associated with oxidative stress throughout the placenta. Although the relationship between spontaneous abortions and some circulating markers of oxidative stress has been evaluated in some animal and human studies, the published data is not uniform and the exact mechanism for the spontaneous expulsion/ retention is not clear. Our earlier reverse transcription-PCR and biochemical studies, performed on various biomarkers of oxidative stress including lipid peroxide/nitric oxide/superoxide dismutase/ glutathione reductase, suggested role of superoxide dismutase in *C. trachomatis*-positive women undergoing spontaneous abortion in the first trimester.

Work done during the year

During the reporting period, the *in situ* expression of Cu, Zn-SOD and Mn-SOD was studied by immunohistochemistry in the endometrium of women throughout the menstrual cycle, and in the endometrial curettage tissue during early pregnancy and in *C. trachomatis* infected spontaneous aborters. The epithelial cells showed a positive immunostaining for Cu, Zn-SOD and Mn-SOD in the endometrium throughout the menstrual cycle and in early pregnancy. In the stroma, weak immunostaining for Cu, Zn-SOD and moderate immunostaining for Mn-SOD were observed in the pre-decidual cells

in the late secretory phase, but no immunostaining was observed in the stromal cells from early proliferative phase to mid-secretory phase. Decidual cells in early pregnancy showed strong immunostaining for Cu, Zn-SOD and Mn-SOD. In curettage tissue from spontaneous aborters diagnosed as *C. trachomatis* positive, moderate immunostaining for Cu, Zn-SOD was observed in the cytotrophoblast while endothelial cells exhibited occasional staining. The cellular distribution of Mn-SOD was similar to Cu, Zn-SOD although the intensity of immunostaining varied and was less intense than that observed with the Cu, Zn-SOD antibody. Occasional syncytial knots showed positive expression for Mn-SOD.

2. IMMUNOMOLECULAR EXPRESSION OF CYCLOOXYGENASES AND PROSTAGLANDIN RECEPTORS IN ENDOMETRIAL CURETTAGE TISSUE OF CHLAMYDIA TRACHOMATIS INFECTED WOMEN DURING SPONTANEOUS ABORTION

Scientific Staff	**:** Dr. Sangita Rastogi, Ms Namita Singh
In collaboration with	**:** Dr. Banashree Das, SJ hospital, New Delhi
Duration	**:** 2008-12

Aims, Objectives & Background

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 and its subsequent conversion to prostaglandin E2 (PGE_2) and PGF2alpha. PGE_2 is induced in the reproductive tract epithelial cells following chlamydial infection. Krausse et al demonstrated that equivalent numbers of *C. trachomatis* per host cell induced a higher PGE_2-response. The amount of synthesized PGE_2 depends on the chlamydial multiplicity of infection. Till date, there is lack of information regarding expression of EP receptor subtypes in human pregnancy. Our hypothesis is to study the differential expression of PG receptors / cyclooxygenases which might be important for regulating uterine activity throughout pregnancy and during spontaneous abortion. The study aims to characterize the expression of cyclooxygenases during prostaglandin

biosynthesis in spontaneously aborted tissues from women found infected with *C. trachomatis*. Patients undergoing MTP were included as controls to study the role of cox-2 in non-pregnant women without chlamydial infection.

Work done during the year

During the reporting period, endometrial curettage tissue samples were further collected by dilatation and evacuation from spontaneous abortion patients (06-12 weeks of gestation) attending Safdarjung hospital, New Delhi. The curettage tissue was histologically examined for the presence of foetal membranes / tissues by H & E and by cytokeratin immunohistochemistry. PCR assay and *in situ* localization of *C. trachomatis* were done for the diagnosis of chlamydial infection in the endometrial curettage tissue. Earlier studies on *in situ* expression of cyclooxygenases and prostaglandin receptors by immunohistochemistry and reverse transcription PCR in the curettage tissue of spontaneous aborters showed that the expression of cycloxygenase-2 and prostaglandin receptor-2 was increased in *C. trachomatis*-infected women.

Quantitative Real time PCR assay was performed on 7000 Real time PCR system (*Applied Biosystems*) in the curettage tissue samples for studying the expression of Cox-1, Cox-2 and Prostaglandin receptors. The reactions were set in duplicates. The assay was standardized for the internal control gene (GAPDH) in tissue samples. Samples showing consistency with GAPDH were selected for real time PCR assay. Threshold cycle (Ct) value was calculated as average Ct target genes for each sample by using SDS software. Ct values obtained after real-time PCR were analyzed. The Cox-2 gene was 1.92 fold upregulated in *C. trachomatis* infected spontaneous aborters (Fig. 1), whereas the Cox-1 gene was 3.2 fold downregulated. The expression of EP-2 gene was also found 1.9 fold up-regulated *C. trachomatis* infected aborters (Fig. 2).

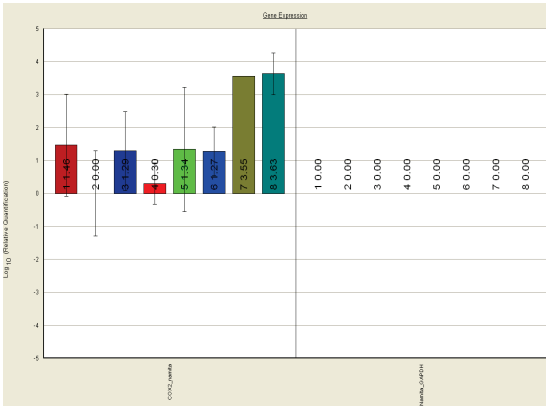


Fig. 1: Fold change in the expression of cox-2 gene in comparison to control.

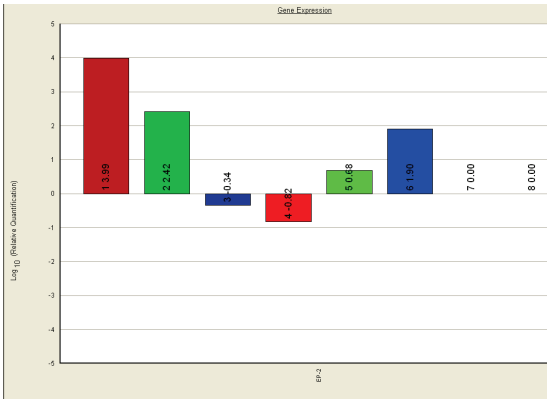


Fig. 2: Fold change in the expression of EP-2 gene in comparison to control.

3. IMMUNOPATHOGENESIS OF REACTIVE ARTHRITIS INDUCED BY CHLAMYDIA TRACHOMATIS

Scientific Staff	:	Dr. Sangita Rastogi, Mr. Praveen Kumar
In collaboration with	:	Brig. (Dr.) DS Bhakuni, Army R & R hospital, New Delhi
Duration	:	2009-13

Aims, Objectives & Background

Reactive arthritis (ReA) is an acute inflammatory joint disease belonging to the group of spondyloarthropathy (SpA). The post-urethritic and post-dysenteric forms of ReA are mainly caused by *Chlamydia trachomatis* and enterobacteria (such as *Yersinia*, *Salmonella*, or *Shigella*), respectively. Epidemiologic work has established *C. trachomatis* as the most common organism leading to ReA in the course of a urogenital infection. However, *C. trachomatis* infection is frequently asymptomatic and while a high prevalence of chlamydial genital infection has been reported from India, there is a paucity of studies on the prevalence of *C. trachomatis* in ReA patients. It is probable that the role of ciReA (*Chlamydia*-induced ReA) is possibly underestimated in our country. Further, studies on the immunopathogenesis of ReA are meagre and data on synovial fluid cytokine

concentration in such patients is contradictory. Hence, the aims of the study are to prospectively screen both ReA as well as undifferentiated spondyloarthropathy (uSpA) patients for intra-articular diagnosis of chlamydial DNA in the synovial fluid by PCR and elucidate the role of Th1/Th2 cytokines in ciReA patients.

Work done during the year

38 patients of SpA constituted the study group. Of these, 16 were categorized as ReA patients according to the ESSG (European Spondyloarthropathy Study Group) criteria while the remaining 22 were uSpA patients. Most patients had an oligoarthritis of the lower limbs with at least one swollen joint (*i.e.* knee synovitis). The mean disease duration was 16.2 months while the male: female ratio was 23:15 in these patients. Patients with a diagnosis of ankylosing spondylitis, psoriatic arthritis or enteric spondyloarthritis were excluded. Blood and synovial fluid sample were also collected from a control group comprising of 9 rheumatoid arthritis (RA) and 6 osteoarthritis (OA) patients.

Diagnosis of intra-articular *C. trachomatis* infection by PCR:

Semi-nested (snPCR) and nested PCR (nPCR) were performed by targeting 16srRNA / MOMP / plasmid genes for the detection of *C. trachomatis* in the synovial fluid of arthritic patients, *viz.:* ReA, uSpA and controls. The overall positivity by PCR for *C. trachomatis* in the study group, *i.e.* ReA / uSpA, was 8/38 (21.9%). Semi-nested PCR for MOMP gene showed the prevalence of *C. trachomatis* in 6/38 (15.7%) ReA / uSpA patients while nested PCR for *C. trachomatis* plasmid gene revealed 7/38 (18.4%) patients to be positive for this microorganism.

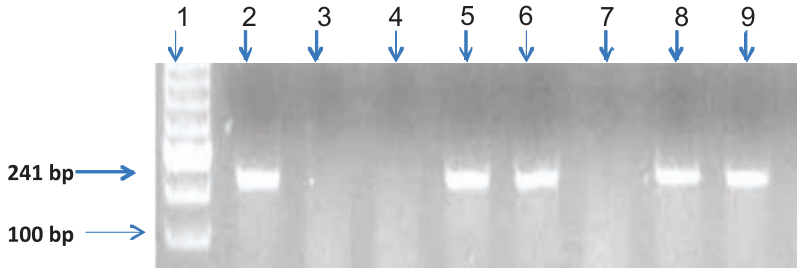


Fig: Arrow shows 241 bp nested PCR product of *C. trachomatis* plasmid gene in ReA patient. Lane 2: positive control; lane 3: negative control; lanes 4-9: clinical samples; lanes 5-6 and 8-9: positive samples.

Detection of anti-*C. trachomatis* antibodies:

The estimation of anti-*C. trachomatis* IgA antibodies was done in ReA and uSpA patients by using a commercial kit (*Savyon Diagnostics, Israel*) as per the manufacturer's guidelines. *C. trachomatis*-specific IgA antibodies were found in the synovial fluid and serum of 12/38 (31.5%) and 6/38 (15.7%) patients, respectively.

Estimation of cytokines:

The level of cytokines, *viz.:* IFN-gamma and IL-4 was determined in the synovial fluid of ReA / uSpA patients with commercial Elisa Ready-Set-Go kits (*eBiosciences, USA*) according to the manufacturers' instructions. Synovial fluid cytokine level of IFN-gamma was found to be significantly higher in patients with ReA / uSpA as compared to the non-inflammatory controls, *viz.:* OA ($p < 0.002$ and $p < 0.001$ respectively). Further, in comparison to OA, the level of IL-4 was significantly increased in patients with ReA / uSpA ($p < 0.001$). The level of IFN-gamma / IL-4 in the synovial fluid from patients with ReA / uSpA was found to be higher in comparison to RA (inflammatory controls); however, this was statistically insignificant. The study is in progress.

LEPROSY

1. CLINICOPATHOLOGICAL DETERMINANTS IN LEPROSY TYPE 1 REACTIONS

Scientific staff	:	Ms Ira Sharma, Dr. LC Singh, Dr. Avninder P Singh
Technical staff	:	Mr. Ishwar Singh
In collaboration with	:	Dr. V Ramesh, Department of Dermatology, Safdarjung hospital, New Delhi. Dr. Neelam Sood, Dr. Rati Makkar, Department of Pathology & Dermatology, DDU hospital, Delhi. Dr. Asha Kubba, Pathologist, Delhi Dermpath laboratory.
Duration	:	2010-13

Aims, Objectives & Background

Despite the prevalence rate of leprosy in India having fallen to 0.74 and it being declared eliminated, it continues to be a cause of significant public health problem and morbidity in endemic regions. The number of new cases detected has not shown a comparable decline despite a gradual fall in the prevalence rate. There is need of focus on identification and prediction of Lepra reactions that are acute inflammatory complications often presenting as medical emergency during the course of treated or untreated leprosy. Unlike Type 2 leprosy reactions, the clinical features of type 1 reactions are not easily recognizable. This accounts for the discrepancy between the clinician and the histopathologist. The aims of the study are as follows:

1. To define and validate key clinical parameters for early diagnosis of leprosy reversal reaction and evaluate clinicopathological discordance.

2. To define and validate standard diagnostic criteria for histopathological diagnosis of T1R with special emphasis on interobserver variability.

3. To study immunohistochemical expression of i-NOS, nuclear factor-kappa B, IFN-gamma and CXCL 10/IP-10 and to study their co-localization in lesional skin using confocal microscopy.

Work done during the year

Since there are no well-described and published criteria for the histological diagnosis and hence confirmation of leprosy type 1 reaction, we correlated the clinical and histopathological findings in these 120 cases of clinically diagnosed lepra T1reaction. Each pathologist blinded to the clinical diagnosis evaluated the histopathological parameters in these 120 skin biopsies separately without knowing the observations of the other pathologist. A Spearmans rank correlation was done to check for interobserver variations. Of a total of 120 cases suspected of T1R, each pathologist (P1-P3) scored the histopathological findings of the skin biopsy into no reaction, probable reaction and definite reaction, the details of which are shown in Tables 1-2.

P1 found definite evidence of reaction in 65/120 (55%) while P3 found the least number 39/113 (34.5%) of definite T1R on histology. All three pathologists observed that epidermal erosion and dermal edema and intragranuloma edema were the most common parameters seen in a T1R. Another most consistent finding seen by all three pathologists was higher than expected lymphocyte accumulation within the granulomas. For studying immunohistochemical expression of i-NOS, NFkB, CXCL10 and CXCR3 in confirmed T1R biopsies versus non-reaction leprosy, we divided the biopsies into 3 groups. Group 1 included BT and BT-T1R, group 2 included BB and BB-T1R while group 3 included BL and BL-T1R. The immunohistochemical scores were then analyzed by determining the Odds ratio and 95% Confidence Interval (CI) for each marker so as to find any statistically significant correlation that can differentiate a reaction from non-reaction. No significant correlations were found in BT /BT-T1R and BL/BL-T1R. However, in BB versus BB-T1R, CXCR3 (p=0.040) and CXCL10 (p=0.049) were statistically significant in distinguishing reaction from non-reaction. Further immunolocalization of these proteins will be determined by immunofluorescent staining and also gene expression of i-NOS and CXCL10 has been standardized. In 3 skin biopsy samples, 2 samples showed upregulation and 1 showed downregulation of i-NOS while all 3 samples showed upregulation of CXCL10.

Table 1: Histological diagnoses of T1R given by pathologists for clinical diagnosis of T1R

S.No	Parameter	P1		P2		P3	
		Sp Coeff	P-value	Sp Coeff	P-value	Sp Coeff	P-value
1	Epidermal erosion	0.601	0.00	0.569	0.00	0.198	0.03
2	Dermal Edema	0.810	0.00	0.743	0.00	0.528	0.00
3	Intragranuloma edema	0.643	0.00	0.722	0.00	0.392	0.00
4	Intragranuloma lymphocytes	0.492	0.00	0.618	0.00	0.322	0.001
5	Giant cell size & number	0.292	0.001	0.432	0.00	0.263	0.005

Table 2: Histological diagnosis of reaction given by each pathologist

Pathologist	No reaction	Probable reaction	Definite reaction	Total number of biopsies viewed
P1	37 (30.8)	18 (15.0)	65 (54.2)	120
P2	28 (23.7)	27 (22.8)	63 (53.3)	118
P3	39 (34.5)	35 31.0)	39 (34.5)	113

Table 3: Immunohistochemical expression of markers comparing reaction vs non-reaction leprosy

Group	Marker	P-value
Group 1 (BT vs BT-R)	i-NOS	0.997
	NF-kB	0-998
	CXCL10	0.11
	CXCR3	0.998
Group 2 (BB vs BB-R)	i-NOS	0.37
	NF-kB	0.79
	CXCL10	**0.05**
	CXCR3	**0.04**
Group 3 (BL vs BL-R)	i-NOS	0.52
	NF-kB	0.990
	CXCL10	0.30
	CXCR3	0.54

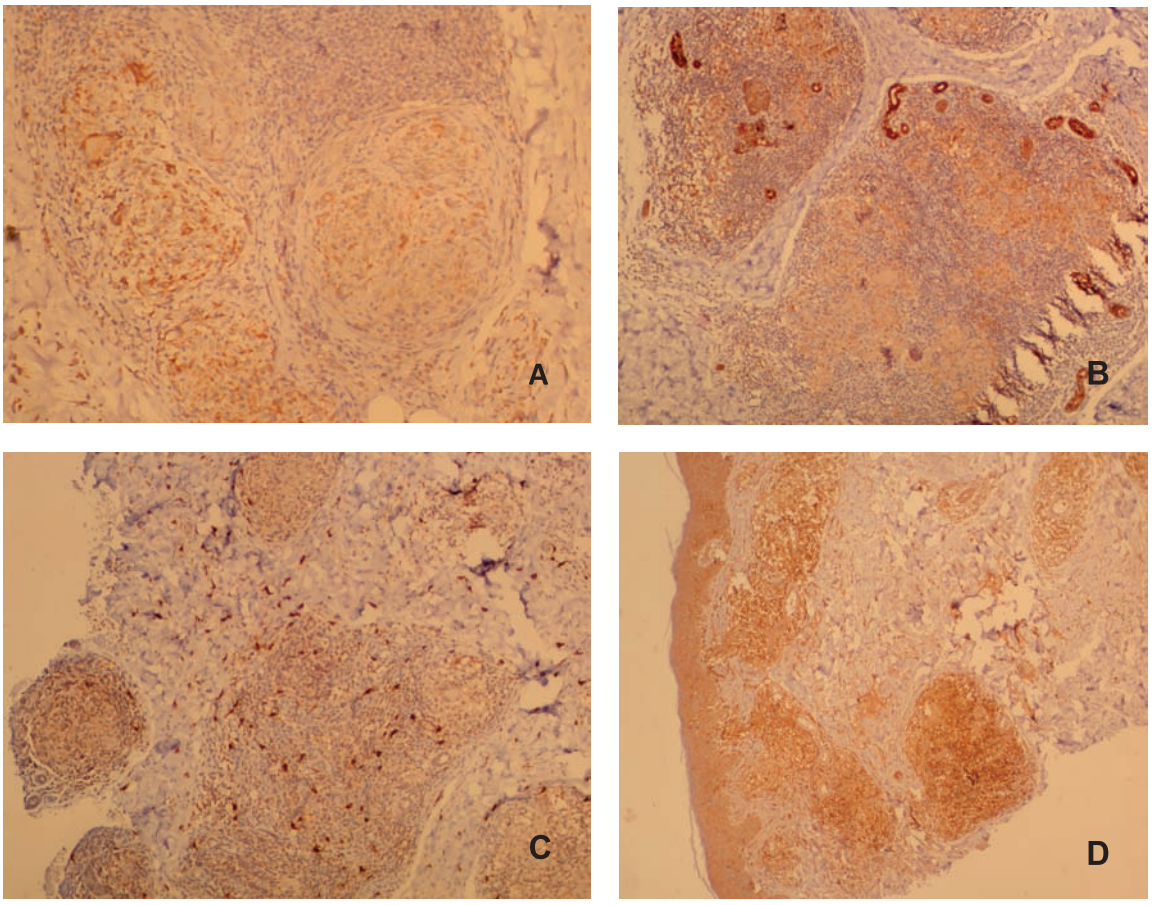


Fig.: Immunohistochemical expression of [A]: iNOS, [B]: CXCL10, [C]: CXCR3, [D]: NFkB in leprosy type 1 reaction in skin biopsies.

TUBERCULOSIS

1. UNDERSTANDING THE UNDERLYING MECHANISM OF MACROPHAGE IMMUNE MODULATION AND IDENTIFICATION OF MARKERS FOR TB TREATMENT END POINT DETERMINATION

Scientific Staff	**:** Dr. Nasreen Z. Ehtesham
In collaboration with	**:** Dr. Seyed E. Hasnain
Duration	**:** 2009-13

Aims, Objectives and Background

Tuberculosis (TB), caused by *Mycobacterium tuberculosis*, is a disease of global significance taking one human life every 15 seconds. The Indian subcontinent is emerging as a global hotspot for the growth and spread of the TB epidemic in recent times, with India alone accounting for 22% of the global burden. Current anti-tubercular treatment (ATT), under the directly observed treatment, short-course (DOTS) regime has documented positive outcomes of successful treatment due to stringent treatment compliance. To prevent relapse or development of drug resistance, in many cases treatment has to be extended for more than six months. Due to lack of sensitive biomarkers for treatment end point determinants, prolonged ATT causes hepatotoxic side effects. There is therefore, an unmet need to identify and develop biomarkers not only for stratification of patients as a function of treatment outcome but also measure relapse rate within the first 2 year of ATT. Availability of such biomarkers for early evaluation of drug response will not only greatly facilitate clinical management of the disease but will also aid in assessing novel anti-tubercular drugs, This is still more needed due to increasing incidence of MDR and XDR TB cases.

In addition to the focus on pathogen, there has been considerable interest about the host defenses in *M. tuberculosis* infection and the associated host-pathogen response. We earlier demonstrated the pro-inflammatory action of human Resistin, a cysteine-rich

secretory adipocytokine. Resistin protein has also been found to be positively associated with several acute inflammatory and chronic diseases such as diabetes, endotoxemia, asthma, severe sepsis or septic shock, atherosclerosis, coronary artery disease, arthritis, malignant tumors, inflammatory bowel disease, non-alcoholic fatty liver disease and chronic kidney disease.

This ability of human resistin to induce a proinflammatory Th1 response was investigated in the context of understanding its role in inducing innate immune response *via* proinflammatory signaling pathways. Simultaneously, we investigated if serum resistin levels could be used to establish the biological co-ordinates of pathogen (*M. tuberculosis*) clearance or treatment outcome. For this, resistin levels were estimated spectrophotometrically, using commercially available human resistin ELISA kit, and compared in patients with TB, their contacts and unrelated healthy controls. Variations in resistin levels, as a function of DOTS treatment were correlated with clinical parameters of recovery namely body weight gain and sputum clearance.

Work done during the year

A. Modulation of macrophage functions following resistin treatments and cytokine profiling:

The human resistin construct in pcDNA was transfected into HEK cell line and the resultant HEK Hres cells were cultured *in vitro* to serve as a source of resistin. The effects of THP 1 derived macrophages exposed to recombinant Hres as well as conditioned medium containing Hres on the cytokine profile were observed.

FACS analyses revealed a direct correlation between increasing concentrations of resistin and expression of TLR2 but not TLR4 (Fig.1). This was NFkB dependent (Fig. 2). Resistin was also found to strongly inhibit the Caspase 8 activation via TLR2 (Fig. 3) as well as inhibition of Caspase activation induced by Staurosporine and ESAT 6 mediated apoptosis (Fig. 4). Resistin was found to stimulate Bcl2 expression, which is not TLR2 dependent (Fig. 5), indicating the possibility of the cytoprotective role of resistin. Caspase 3/7 have also found to been inhibited by Resistin.

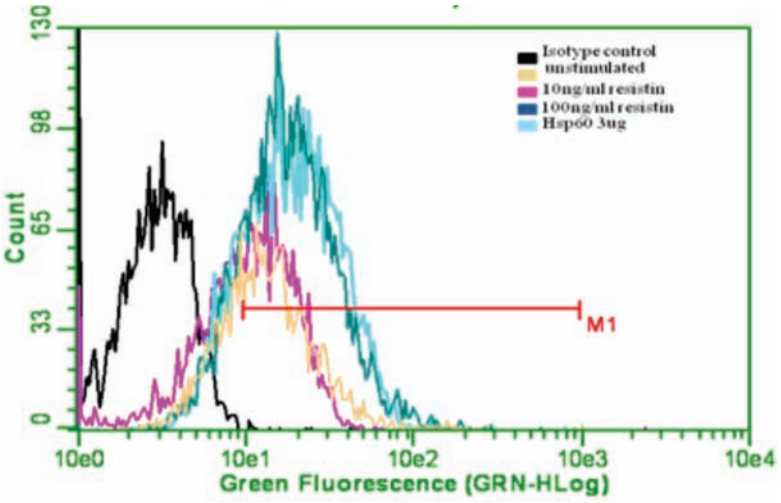


Fig. 1: Resistin stimulates TLR 2. THP1 cells were stimulated with resistin and surface expression was assayed by FACS analysis using TLR2 antibody. Hsp60 was used as control.

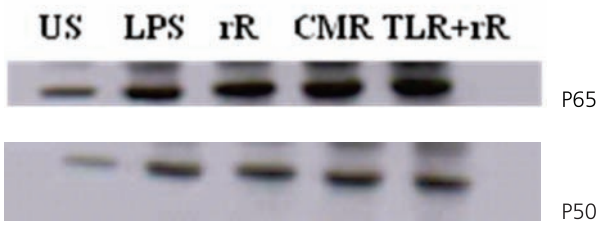


Fig. 2: Resistin mediated NF-Kb (p65 and p50) stimulation is TLR2 independent

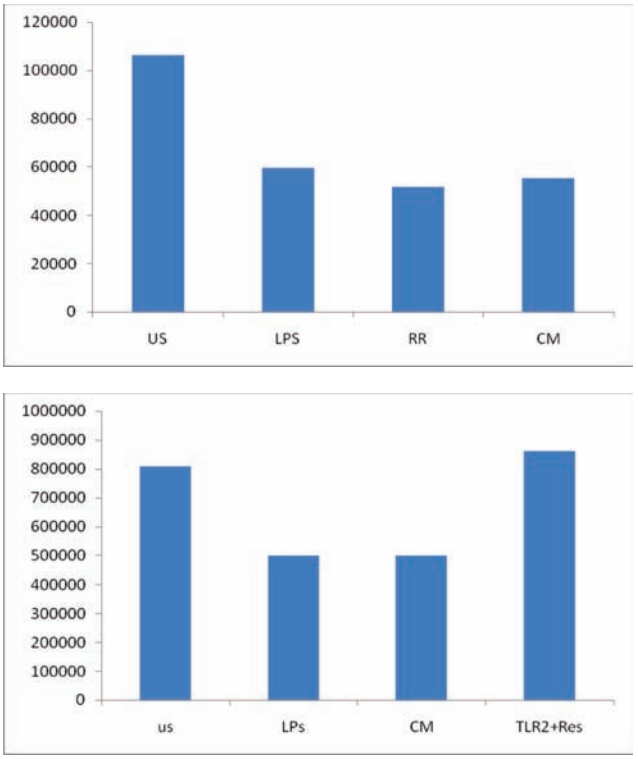


Fig. 3: Resistin inhibits the activation of caspase 8 in a TLR2 dependant manner.

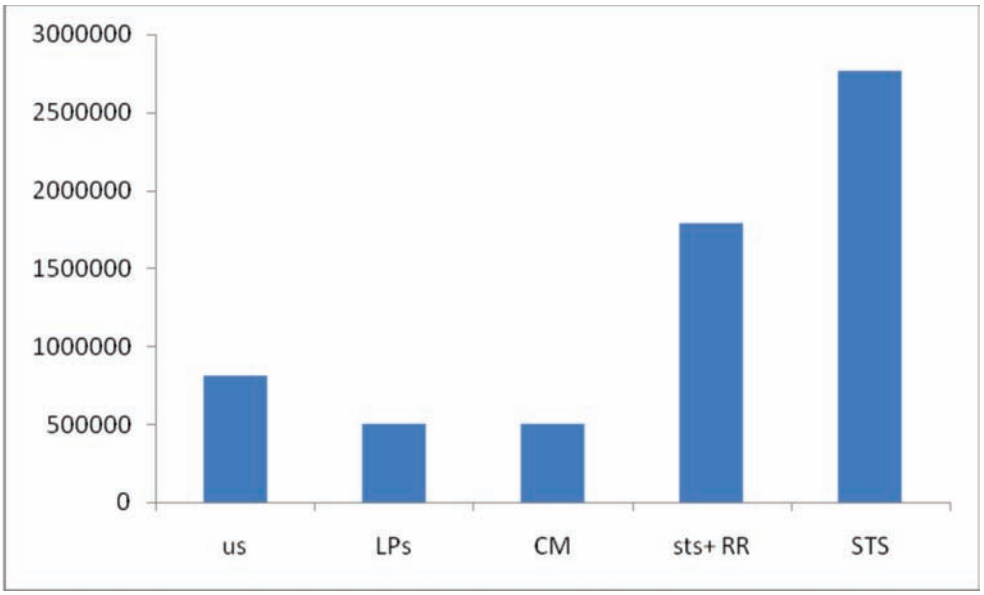


Fig. 4: Resistin downregulates staurosporine induce caspase 8 activation.

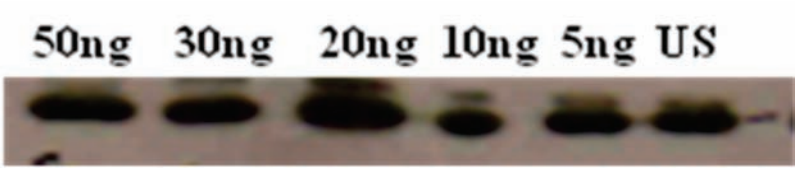


Fig. 5: Dose dependant increase in BCL2 expression following Resistin treatment

B. Identification of a marker for treatment end point determinants:

Serum resistin levels were estimated in contacts and healthy controls at the time of enrollment whereas in patients, at various time points during anti-tubercular treatment [0 month (T0), 2 months (T2), 4 months (T4) and 6 months (T6)] (Fig. 6). Mean value of serum resistin levels (Mean±SD) in TB patients was 25.74±9.45 ng/ml, in contacts 19.61±7.88 ng/ml and in healthy controls 6.54±2.6 ng/ml (Table 1). Resistin level in TB patients (T0) ranged from 3.92 to 40.63 ng/ml, in contact the levels ranged from 3.75 to 38.18 ng/ml and in healthy controls the levels were 0.64 to 10.78 ng/ml. Mean value of serum resistin level in patient (T0) was higher than the healthy controls ($P < 0.001$) and contacts ($P < 0.001$) (Table 1). Mean value of serum resistin level in contacts was higher than the healthy controls ($P < 0.001$).

Resistin level and bacterial load: Pulmonary tuberculosis patients were diagnosed, classified and evaluated on the basis of sputum smear examination. The severity of the disease was assessed on the basis of bacterial load in sputum. They were classified into 3 groups in the present study before ATT namely 1+ (27 patients), 2+ (12 patients) and 3+ (9 patients). The mean resistin value of 1+ is 24.99±10.013 ng/ml, 2+ is 25.88±8.495 ng/ml and 3+ is 27.84±9.642 ng/ml (Fig. 7). Even though, the resistin value appears to increase with bacterial load, ANOVA of the data showed no statistical significance ($P = 0.74$).

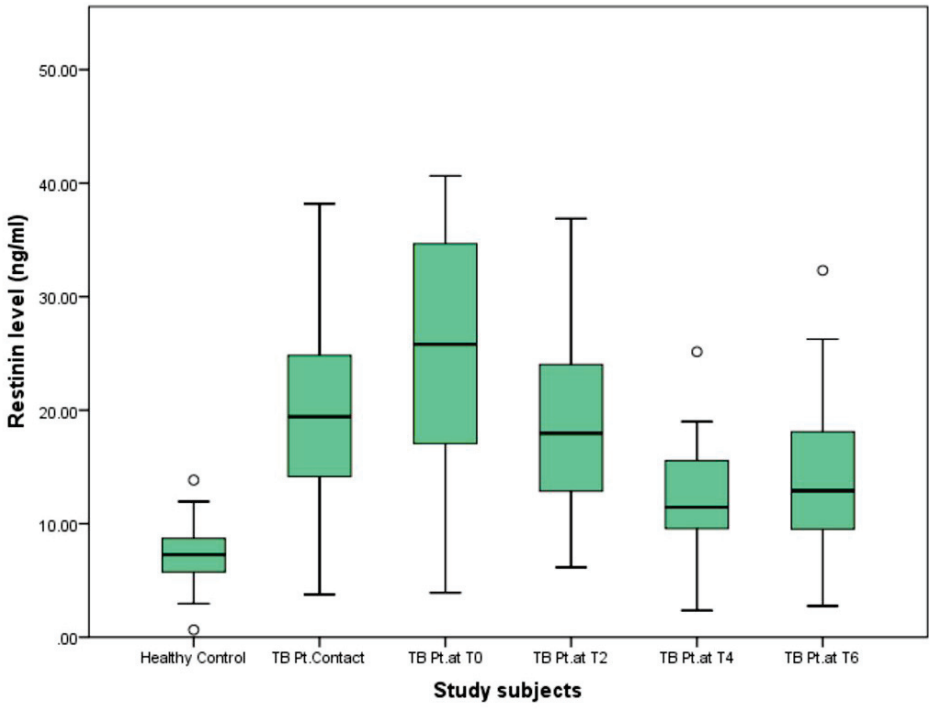


Fig. 6: Resistin levels show significant decline as a function of anti-tuberculosis treatment (ATT) in TB patients. Comparison of the circulating resistin levels in, TB Pt at T0= serum sample taken before the start of treatment; TB Pt at T2= serum sample taken after 2 months; TB Pt at T4= serum sample taken after 4 month; TB Pt at T6= serum sample taken after 6 months after start of treatment; the healthy control and TB patients contacts using the box plot analysis.

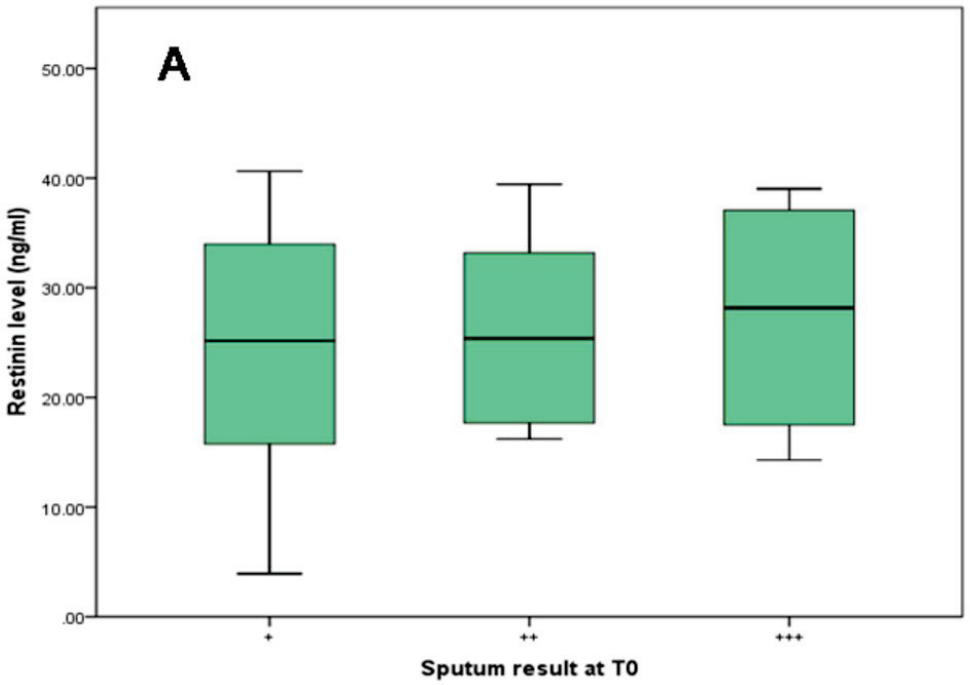
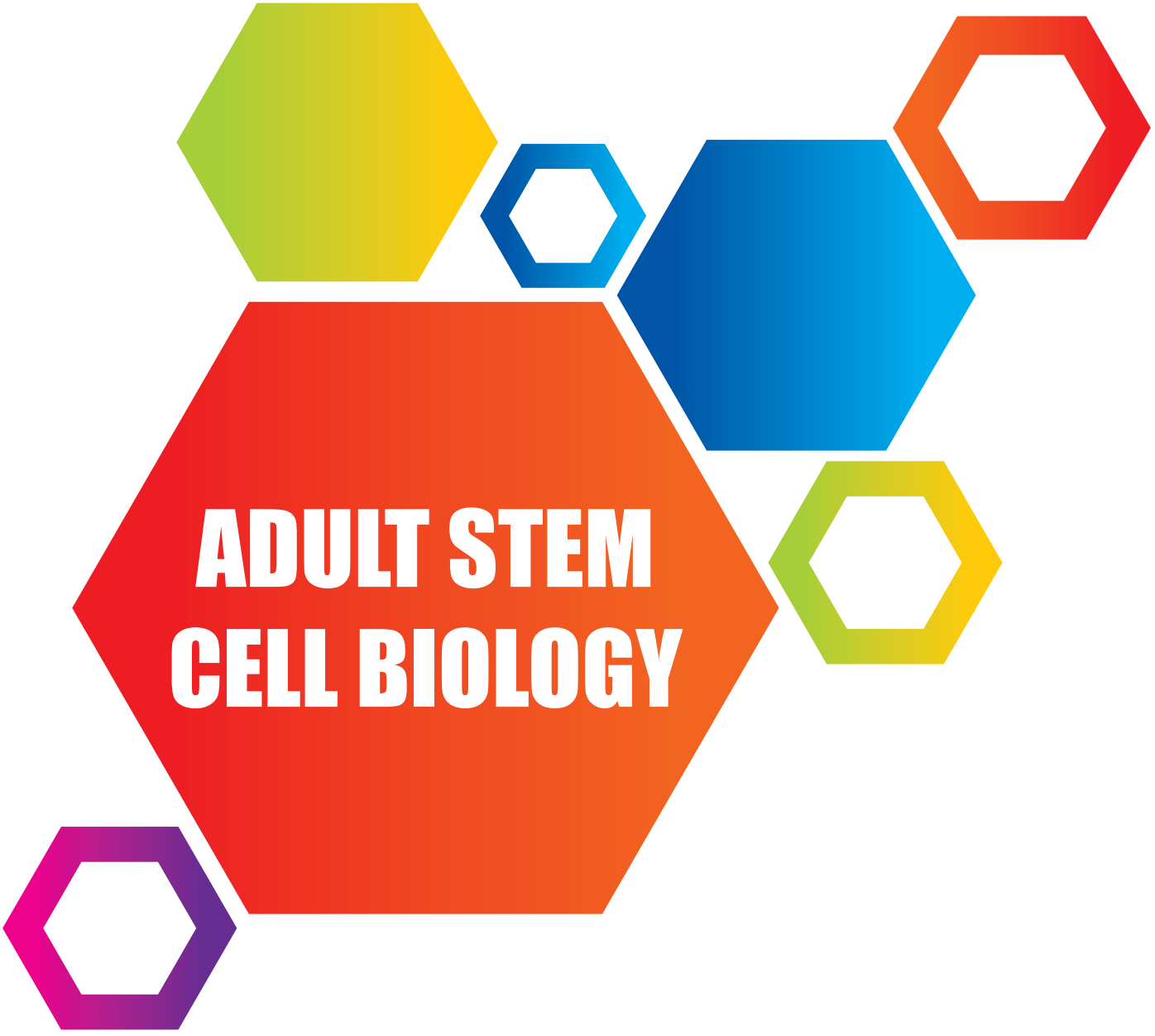


Fig.7: Resistin levels increase as a function of bacterial load. Patients reporting for pulmonary TB treatment were clinically assessed for TB disease severity based on bacterial load in sputum. Patients sputum smear grading was done as (1+) group, (2+) group and (3+) group on the basis of bacilli load.

Given the highly encouraging results described above, we would like to convert this proinflammatory host protein into a marker specific for TB, by developing a multiplex system containing *M. tuberculosis* specific markers. It would also be interesting to expand this study further to assess the diagnostic and prognostic potential of serum resistin level in relapse and extra-pulmonary TB cases. Development of such specific marker system will also be useful in providing early assessment of novel drugs and vaccine against TB.


ADULT STEM
CELL BIOLOGY

ADULT STEM CELL BIOLOGY

1. A NOVEL ARITHMETIC APPROACH FOR FOOL-PROOF PRODUCTION OF GROWTH ARREST IN 3T3 CELLS SUITABLE FOR HUMAN EPIDERMAL CULTURE

Scientific Staff	: Dr. LK Yerneni, Mr. Bijender, Mr. Rishi Man Chugh (SRF, ICMR Project)
Duration	: 2010-13

Aims, Objectives & Background

The present investigation employs SWISS 3T3 cells adopting a strategy of developing a culture system for growing Cultured Epithelial Autografts, which is similar to our earlier innovative process of growth arrest in NIH 3T3 feeder cells (Yerneni & Kumar, 2009; A culture system for the growth of stem cells. Indian Patent File No.2086/DEL/2009, Filing date 7/10/2009, Publication date 13/8/2010, Issue 33/2010, p 24092, International Classification No: C12N). In brief, the culture system involves attainment of optimal growth arrest employing arithmetically derived doses of mitomycin C. We intend to use the Swiss Albino 3T3 cell line (ATCC Cat No.CCL-92), which is the popular choice as feeders for stem cells, for the proposed future clinical application studies at our institute. So far we have standardized a validated 2-tier banking protocol for the feeders, cell density dependant variation in the cell extinction (growth reduction curves), in response to mitomycin C and arithmetic derivation of probable numerical doses for appropriate volumetric titrations to demonstrate their static, inhibitory and toxic out comes. The study objectives are as follows:

1. To find out correct exposure conditions for a fool-proof mitomycin C induction of 3T3 feeder cell attenuation through arithmetic derivation of effective concentration based on cell number.

2. To verify cell proliferative influence of such fool-proof growth arrested 3T3 cells on human epidermal keratinocytes.

3. To characterize the human epidermal keratinocytes and epidermal sheets cultivated using such fool-proof growth arrested 3T3 cells.

Work done during the year

Experiments were conducted to arrest of Swiss 3T3 to verify the same property of dose-per-cell dependency of mitomycin C (MMC) on growth arrest as it was shown with NIH 3T3 earlier (Yerneni & Kumar 2009, Indian Patent No.2086/DEL/2009). The differential influence of such variedly growth arrested feeders was tested using Keratinocyte-feeder co-culture system.

Attenuation Protocol for Swiss 3T3 cells: In brief, the 3T3 fibroblasts, at a density of 23,838 ±714 cells per cm^2 were exposed to a 2-hour pulse of MMC at concentrations of A, B, C & D which are concentrations [X], [X+1], [X+2] and [2(X+2)] μg/ml, respectively (as reported in Annual Report 2010-11). Concentrations B & C (inhibitory) were further subdivided into 4 arithmetically derived doses viz., p, q, r & s which are [x], [5x], [10x] & [30x] $\mu g/10^6$ cells, respectively, whereas concentrations A (static) and D (Toxic) were tested at a minimal dose of 'p' and were included in the study for comparison. At the end of exposure, the cells were detached with trypsin (0.1%)-EDTA (0.2%) solution, counted and replated into 24 well plates at a density of 7 x 10^3 cells / cm^2. Subsequently, the cells were trypsinized, stained with trypan blue and viable cells were counted in Neubauer chamber at days 3, 6, 9, 12 and 20. Each experiment was performed in triplicate and repeated at least thrice.

Co-culture of human keratinocytes with attenuated feeders:

In order to correlate the differential cell extinction curves of 3T3, as influenced by concentration-dose combinations, with keratinocyte cell proliferation, viable cell counts were performed on separately collected 3T3 and keratinocytes at intervals of 3 days until 12 days in a co-culture system. The seeding densities employed for feeders and keratinocytes for all the co-culture experiments were 15000 and 5000 cells per well, respectively. For separate cell collection, the cultures were first treated with 0.02% EDTA

to selectively detach 3T3 cells which were collected into PBS followed by collection of keratinocytes into separate vials after detaching them using 0.25% trypsin + 0.03% EDTA solution containing 0.025% glucose.

Statistics: The data was subjected to analytical protocol (Yerneni and Kumar, 2009) to verify concentration-dose influence of mitomycin C on 3T3 cells and keratinocyte growth. Line diagrams were plotted with various concentration-dose combinations on x-axis and cell numbers representing 3T3 on days 3, 6, 9 and 12, respectively, on y-axis. Similarly, scatter plots for each of the most effective concentration-dose combinations (B and C) were constructed with time (Days) on x-axis and all the triplicate cell extinction values for 3T3 on each day were plotted on y-axis. Linear trend lines were plotted by calculating the least squares fit through cell count data points using regression analysis and r squared values were calculated.

Growth curves for various treatment groups of 3T3 and the respective keratinocytes were plotted with viable cell number on y-axis and time (Days) on x-axis. The influence of different concentration-dose combinations on cell numbers of 3T3/ keratinocytes was statistically analyzed by Student's t-Test. The correlation of change in cell numbers of 3T3 and keratinocytes over a period of 12 days after different concentration-dose combinations were compared by regression analysis.

Significant Results:

Attenuation Protocol with Swiss 3T3 cells:

There was a marked 3T3 cell extinction differential immediately after 2-hour pulse exposure to MMC at various concentration-dose combinations (Figure1). The viable cell count before after 2-hour pulse with various concentration-dose combinations was significant in all the groups. It is noteworthy that the values were significantly ($P<0.05$) different among all concentration-dose combinations within the concentrations B and C, except between B-p and B-q, indicating the initiation of differential feeder survival outcome with arithmetic doses at a time point as early as two hours.

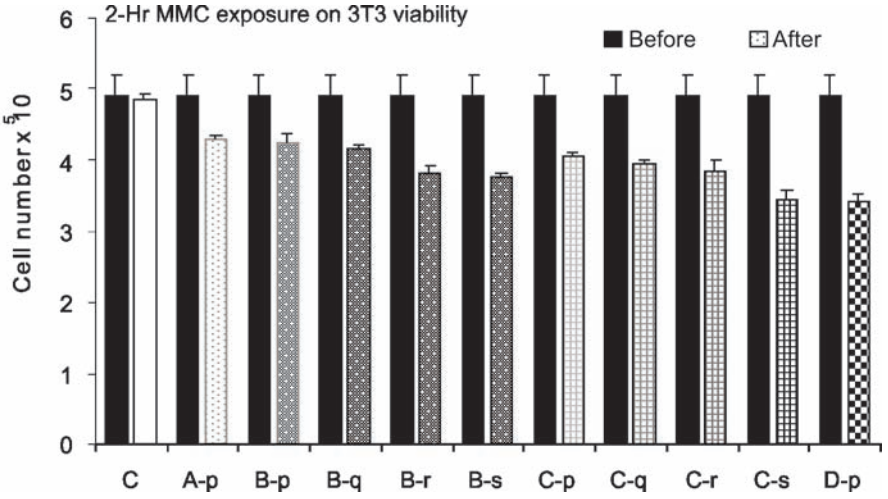


Fig. 1: Swiss 3T3 cell viability immediately after 2 hour pulse with mitomycin C (MMC) at concentrations A,B, C & D and doses p,q,r & s.

Out of a myriad of arithmetically derived doses tested, intermediate concentrations (B & C) yielded maximal dose dependant significant differences in cell extinction rates within each of these concentrations as compared to the lower A (least inhibitory) and higher D (highly toxic) (Fig. 2).

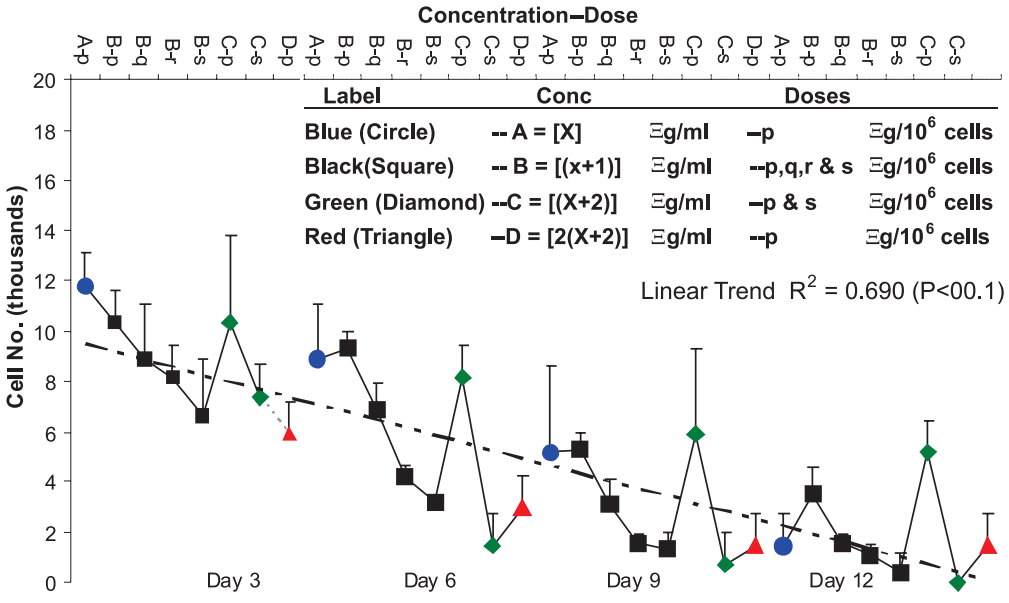


Fig. 2: Influence of various arithmetically derived concentration-dose combinations of mitomycin C on periodic cellular extinctions of Swiss 3T3 cells.

Hence, for further studies using co-culture with epidermal keratinocytes, only one dose each was included for these concentrations.

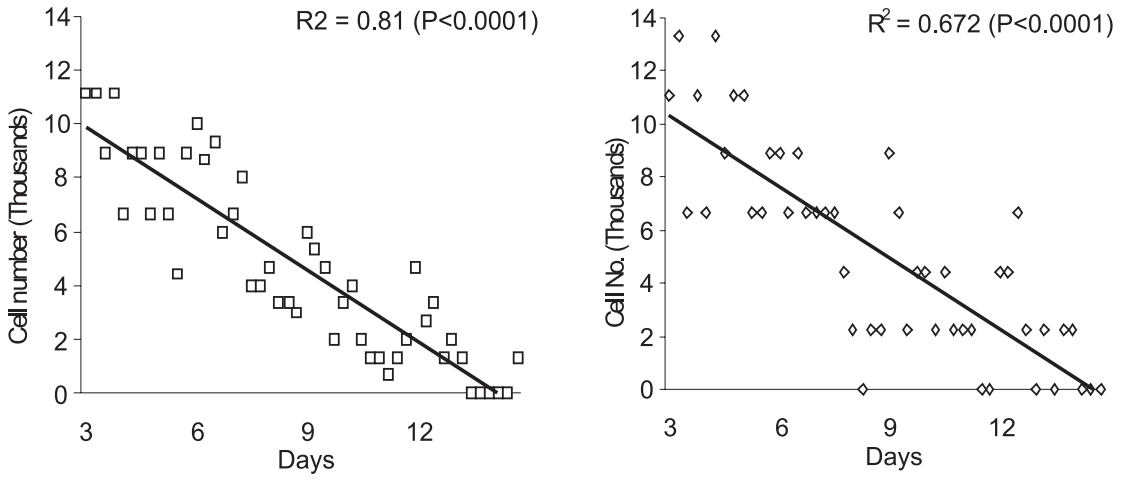


Fig. 3: Viable Cell counts of Swiss 3T3 cells treated with 4 doses (p, q, r & s μg/10^6 cells) of mitomycin C at the concentration of C [(X+1)] μg/ml (Left) and B (X+2) μg/ml MMC (Right).

The regression analysis on extinction rates induced by all the 4 doses of mitomycin C under concentrations of B and C (Figure 3), respectively, over 12 day period showed highly significant fall in cell number proportionate to the increase in dose.

Co-culture of human keratinocytes with attenuated feeders:

Similar to the (arithmetically calculated) dose dependent cellular extinction of Swiss 3T3 cells, the human epidermal keratinocytes plated along with the feeders showed dose dependent cell proliferation in a negatively correlated fashion (Figs. 4-5).

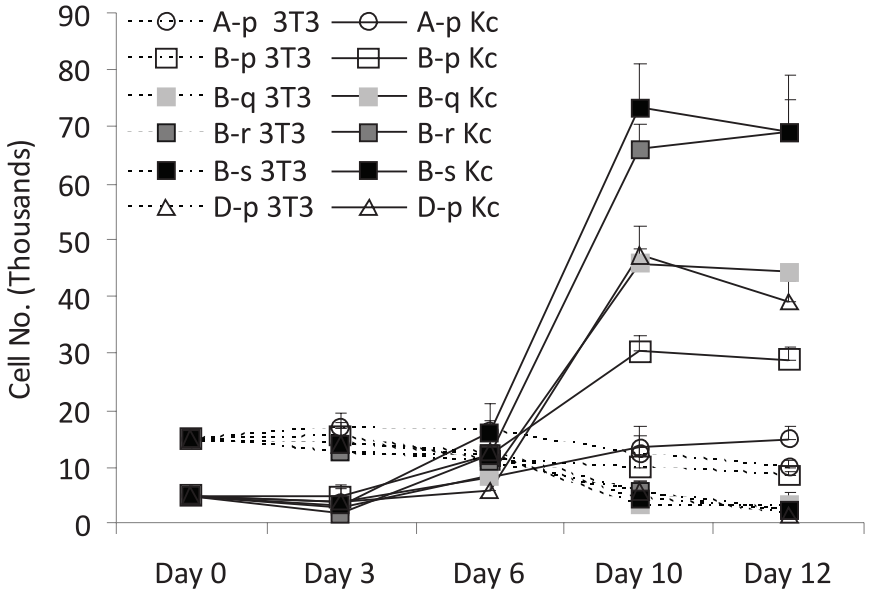


Fig. 4: Differential Keratinocyte proliferation over differentially growth arrested Swiss 3T3 feeder cells using 4 doses (p, q, r & s μg/10^6 cells) of Mitomycin C at the concentration of B (X+1) μg/ml mitomycin C. The lower (A) and higher (D) concentrations at dose 'p' were for comparisons.

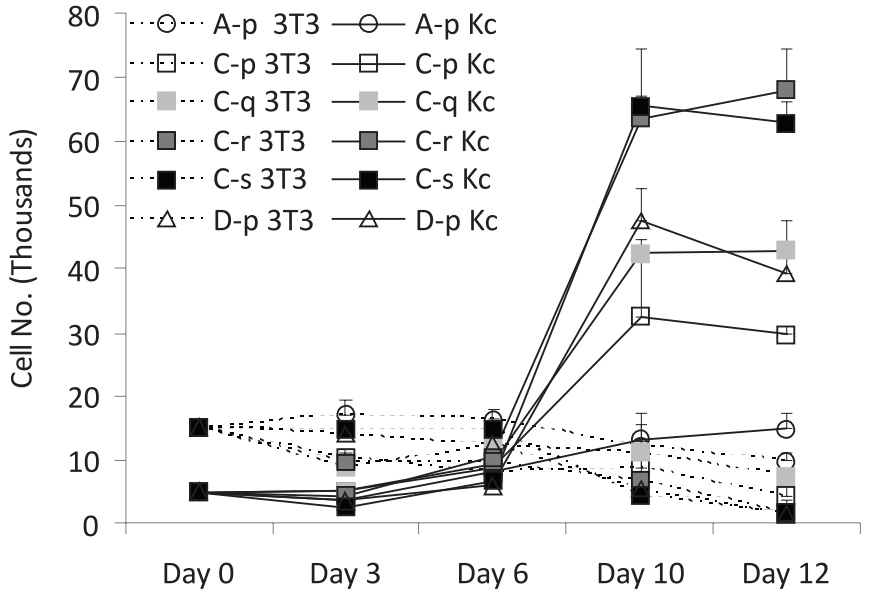


Fig. 5: Differential Keratinocyte proliferation over differentially growth arrested Swiss 3T3 feeder cells using 4 doses (p, q, r & s μg/10^6 cells) of Mitomycin C at the concentration of C (X+2) μg/ml mitomycin C. The lower (A) and higher (D) concentrations at dose 'p' were for comparisons.

Conclusion: It is thus summarized that the mitomycin C induced growth arrest is not only concentration-dependent, but also on the arithmetically derived doses employed, more particularly, in the range of effective but not too toxic doses and this could perhaps be the reason behind a number of contradictory reports in scientific literature on the efficacy of this method compared to γ-irradiation. It thus became possible to modulate the life-span and perhaps the potential expression profile of 3T3 cells through differential induction of growth arrest by differential dosing protocol which resulted in the optimization of keratinocyte cell proliferation. Similar approach has already been demonstrated by us employing dose modulation method in a co-culture system comprising of NIH 3T3 cells as feeders and human epidermal keratinocytes as target adult stem cell population (Yerneni & Kumar 2009).

Potential for a new patent: A New Invention Report highlighting these results obtained so far in the present investigation has been filed with the IPR division of ICMR for assessment towards filing a new patent.

Future course of action: The arithmetically derived dosimetric approach as tested by the volumetric titrations achieving optimization of keratinocyte cell proliferation in co-culture system further requires additional confirmatory tests like colony forming efficiency, clonal analysis using Barrandon & Green 1987, or collagen type IV adherence assay (Kim et al 2004). This will be followed by the production and characterization of cultured epithelial autografts during the third year of the project employing the appropriately growth arrested feeder cells as described in this report.

A recently marketed anti-feeder antibody has been tested in our laboratory on Swiss 3T3 (Fig. 6) to enable quantify target cell contamination with feeders. Further testing of this technique in the co-culture system is being undertaken to validate the specificity. Additionally, it is also being planned to evaluate the differential in post-mitomycin C-exposure cell extinction, whether or not there is involvement of apoptotic pathway. This has been standardized employing fluorescence Pan-Caspase enzyme histo-chemistry using FLICA reagent after staurosporine induced apoptosis in Swiss 3T3 cells (Fig. 7).

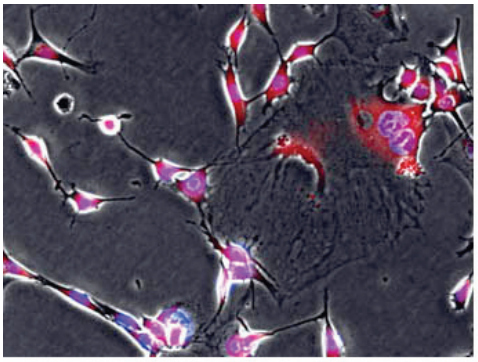


Figure 6. Demonstration of Swiss 3T3 cells using anti-feeder antibody showing peri-nuclear fluorescence (purple to red). Nucleus was counter stained with Hoechst 33258.

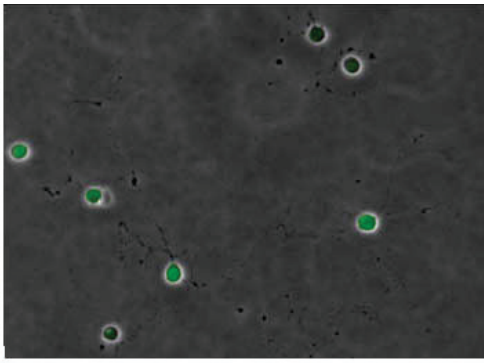


Figure 7. Visualization of Pan-Caspases by enzyme histo-chemistry using green fluorescent FLICA in apoptotic Swiss 3T3 cells induced by 2μM staurosporine.

Merged phase contrast-fluorescence images.

2. STUDIES ON EPITHELIAL-MESENCHYMAL INTERACTIONS USING HUMAN EPIDERMAL KERATINOCYTE STEM CELLS AND INNOVATIVELY GROWTH-ARRESTED FIBROBLAST FEEDERS IN 3-D COLLAGEN MODEL

Scientific Staff	**:** Dr. LK Yerneni, Mr. Madhusudan Chaturvedi (SRF)
Duration	**:** 2010-13

Aims, Objectives & Background

This study has been aimed at understanding the crucial mechanisms of differential growth stimulation of keratinocytes achieved by modulating the feeder cell growth arrest employing mitomycin C (MMC) with an innovative dosimetric protocol. On the basis of our previous experimental results filed as a patent (Yerneni & Kumar, 2009; a culture system for the growth of stem cells. Indian Patent File No.2086/DEL/2009), it is hypothesized that the demonstrated varied extent of attenuation of 3T3 fibroblasts

through manipulation of arithmetically derived numerical doses of mitomycin C (MMC) resulting in differential stimulation of epidermal keratinocyte proliferation could be the consequence of altered fibroblast-keratinocyte interactions that probably bring about qualitative and quantitative differences in epidermal morphogenesis *in vitro*. Fibroblasts from different body sites display different functional properties which may affect their suitability for dermal substitutes (Nolte et al 2008, Cells Tissues Organs.187:165-76) and the differentially growth arrested fibroblasts may similarly be functionally diverse exerting varied responses on keratinocytes. The research work could possibly lead to the formation of unique organotypic skin models with reproducibly varied characteristics suitable for basic and applied research in the field of epidermal regeneration in wound healing. The objectives of the study are as follows:

1. To verify if the differentially growth arrested fibroblast (mesenchymal) feeders through employment of numerical doses of MMC as per the innovative technique, bring about varied growth extinction curves in an organotypic dermal-equivalent culture system.

2. To compare such varied mesenchymal outcomes with the growth of epidermis over the dermal-equivalent consisting of variedly growth arrested 3T3 fibroblasts produced experimentally by employing various numerical doses of MMC.

3. To characterize such variedly produced whole skin-equivalents to establish differential mesenchymal - epithelial interactions that supposedly result through manipulation of MMC dosing.

Work done during the year

3-D Collagen based Dermal Equivalents (DE) were constructed as per Stark et al 2004. Swiss 3T3 cells were differentially growth arrested employing our novel MMC protocol (Yerneni & Kumar 2009, Indian Patent No.2086/DEL/2009) and their cell extinction rates in DE and routine 2-D culture, were estimated. In brief, the 3T3 fibroblasts were exposed to a 2-hour pulse of MMC at concentrations of A, B, C & D which are concentrations [X], [X+1], [X+2] and [2(X+2)] μg/ml, respectively. Each of these, based on previously reported 2-D cell density titration experiments, were further subdivided into 4 arithmetically derived doses viz., p, q, r & s which are [x], [5x], [10x] & [30x] $\mu g/10^6$

cells, respectively. However, the concentrations of A & D were tested at a minimal dose of 'p', while B & C were subdivided into a minimal dose of 'p' and maximal dose of 's' each, for 3-D experiments since only these doses expressed maximal extinction differential in 2-D culture. The cells were subsequently seeded into DE at a density of 3.3 x 10^5 cells per cm^3.

The un-attenuated fibroblasts exposed to MMC vehicle (HBES solution) seeded similarly served as control. The results indicated varied cell extinction patterns in a concentration as well as dose-dependant manner similar to the 2-D experiments (Fig. 1).

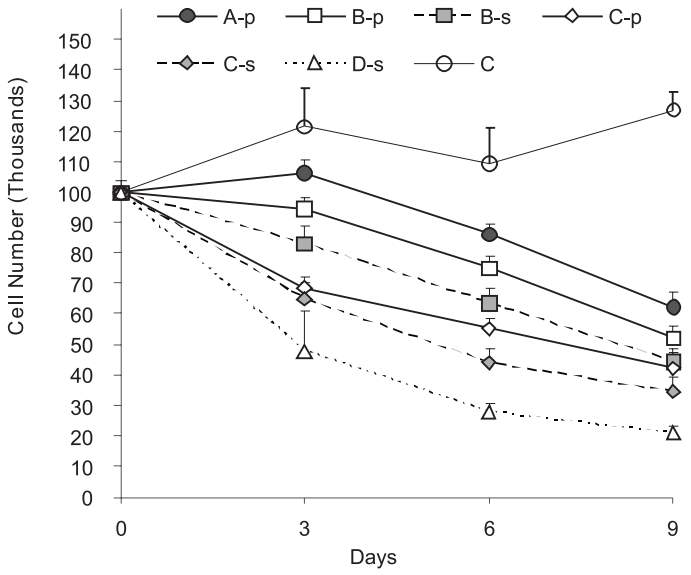


Fig. 1: Influence of various arithmetically derived concentration-dose combinations of mitomycin C on periodic cellular extinctions of Swiss 3T3 cells in collagen gel dermal equivalent.

Additionally during the current reporting year, the basic methodology of preparing the organotypic co-culture skin model consisting of dermal equivalent consisting of 3T3 fibroblasts infused into Type-I collagen gels and epithelialization of the same using human epidermal keratinocytes has been accomplished (Figure 2). This model is currently being fine tuned to make the epithelial tissue fully mature at air-liquid interface for evaluating the action of the experimentally produced various stocks of growth arrested 3T3 cells on epithelialization.

Conclusions: The differentially growth arrested 3T3 cells have exhibited periodic cell extinction patterns similar to those observed with 2-D culture technique.

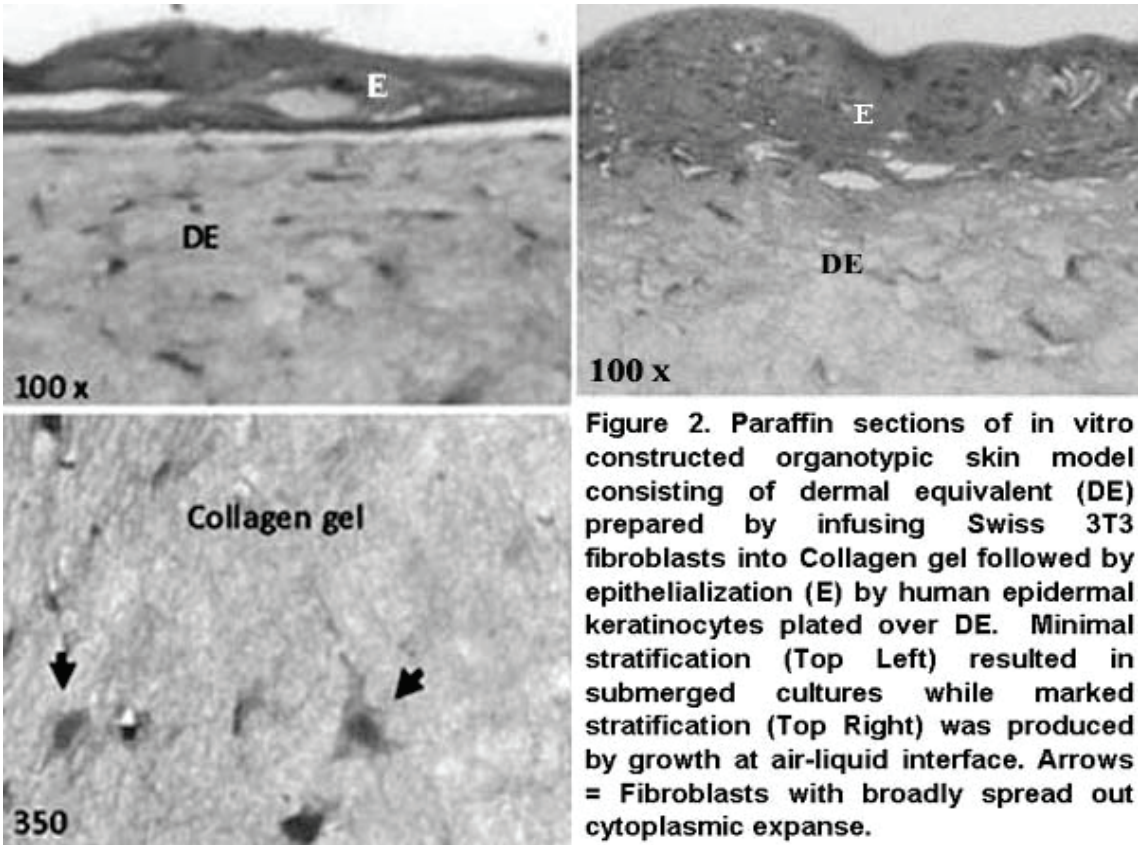


Figure 2. Paraffin sections of in vitro constructed organotypic skin model consisting of dermal equivalent (DE) prepared by infusing Swiss 3T3 fibroblasts into Collagen gel followed by epithelialization (E) by human epidermal keratinocytes plated over DE. Minimal stratification (Top Left) resulted in submerged cultures while marked stratification (Top Right) was produced by growth at air-liquid interface. Arrows = Fibroblasts with broadly spread out cytoplasmic expanse.


ENVIRONMENTAL
TOXICOLOGY

ENVIRONMENTAL TOXICOLOGY

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

Scientific Staff	:	Dr. Arun Kumar Jain, Dr. Nida Akhtar, Mr. Shashi N Kumar
In collaboration with	:	Dr. SK Sharma & Dr. AM Khan, RMRC, Dibrugarh. Dr. Deepa Borgohain, Assam Medical College, Dibrugarh. Dr. Sudha Salhan, SJH, New Delhi.
Technical Staff	:	Mr. Manoj
Duration	:	2008-11

Aims, Objectives & Background

India is one of the major tea producing countries with the north-eastern states accounting for more than 50% of total tea production. It is reported that over 300 species of arthropods and 58 species of fungi infest tea cultivation in India alone necessitating the continuous application of heavy doses of several pesticides.

Every year, about 3 million people are poisoned around the world and 200,000 die from pesticide. Annually, an estimated 25 million workers suffer from pesticide poisoning throughout the world. In developing economies such as India, farmers and agricultural workers are exposed to pesticides directly at the time of mixing and spraying these pesticides. 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. Recently, several NGOs have raised concerns about pesticide contamination of common food stuff, fruits and vegetables. Present study has been designed to assess the pesticide exposure of tea garden workers in north-eastern states of India using placenta and blood of women.

Work done during the year

Study Population:

The study population comprised of the following groups.

1. **Exposed Individuals**:

   a. Women involved in tea farming activities in Dibrugarh (TGW, n=89)

   b. Women involved in agricultural activities other than tea farming in Dibrugarh (AW, n=19)

2. **Unexposed Individuals**:

   a. Women in Dibrugarh region not involved in any agricultural or tea farming activities (NTGW, n=59).

      These included pregnant women admitted / coming for delivery in Assam Medical College, Dibrugarh, and

   b. Women from urban Delhi region not involved in any agricultural or tea farming activities (UHW, n=50).

      These included pregnant women from Delhi NCR admitted / coming for delivery in Safdarjung hospital, New Delhi.

The sample population was screened for inclusion as well as exclusion criterion and relevant portion of proforma questionnaire were filled. Samples of placenta and blood (maternal and cord) were collected at the time of delivery.

Analysis

Initially, a cocktail of about 60 commonly used pollutants consisting of organochlorine, organophosphorus, pyrethroid, herbicide, fungicide and carbamate pesticides as well as poly-aromatic hydrocarbons were selected for multi-residue analysis and the analytical conditions for HPLC were optimized and standardized so as to distinctly separate the selected pollutants.

The completion of the qualitative detection of pesticides was reported in the last report. During the year under report, attempts were made to quantify the pesticides detected. For this purpose, multiple concentrations (ranging from 100 ppm to 0.001 ppm) of different pesticide standards were made and were injected into GC and HPLC columns. The analysis was carried out using standardized protocol and repeated three times. Standard calibration curves were drawn between pesticide concentration and area under the peak. The curves were fitted for linear regression, regression coefficient followed by calculation of regression equation which was later used for determination of pesticide level in the samples. Figs. 1-2 show standard calibration curve for acephate and imidacloprid by HPLC.

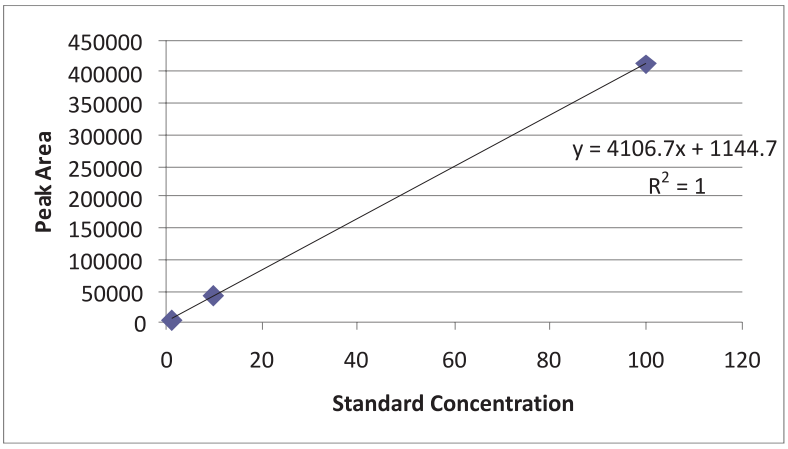


Fig. 1: Standard Calibration Curve for Acephate by HPLC

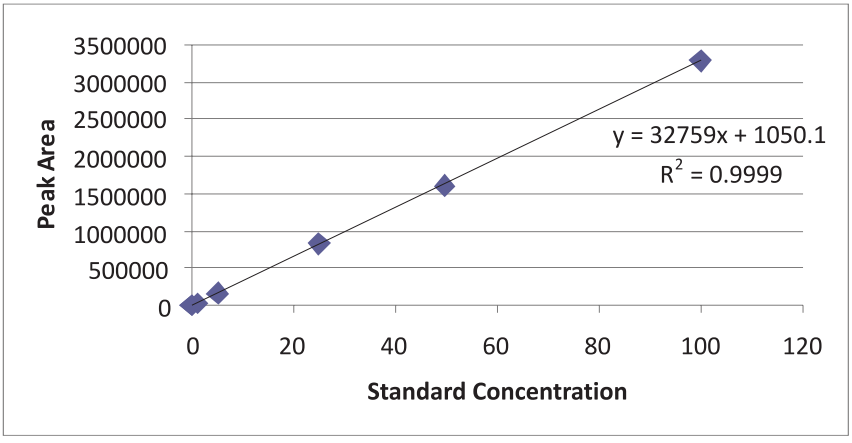


Fig. 2: Standard Calibration Curve (HPLC) for Imidacloprid

Quantification of Pesticides in Dibrugarh and Delhi subjects:

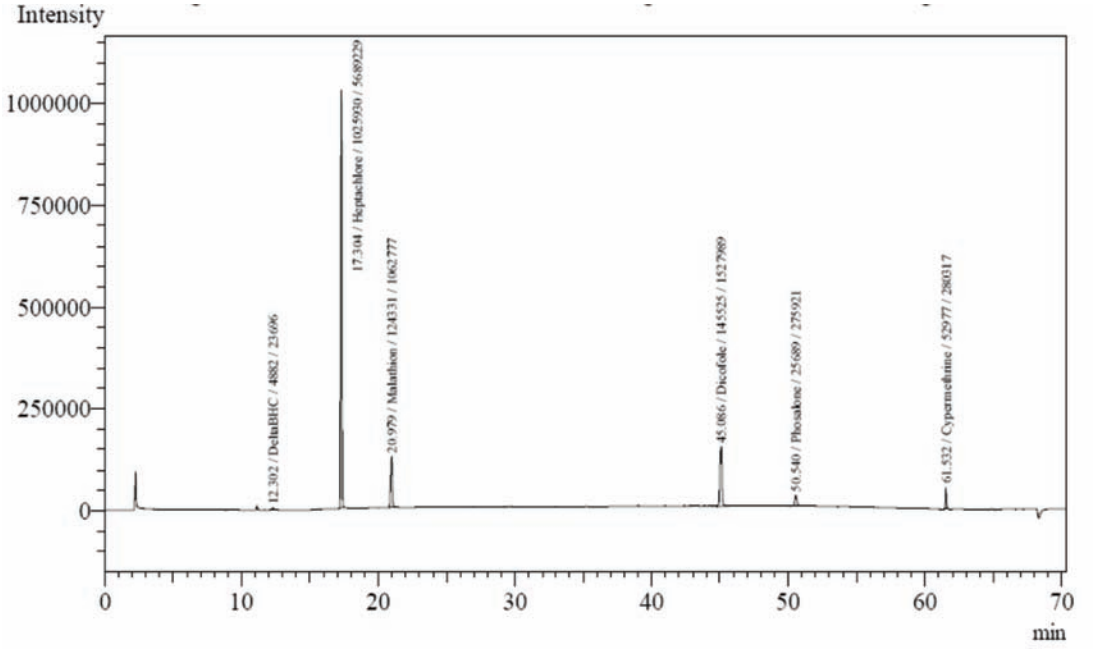
Analysis of the GC / HPLC chromatograms obtained from placenta and blood extracts showed the separation of different pesticides in the form of multiple peaks (Figs. 3-6). Some of these peaks have been integrated and identified with comparison of the RT of the standards pesticides and quantified also on the basis of standard calibration curve. The findings of our study revealed that apart from the exposed population, the pesticides have also been observed in directly un-exposed housewives who might have got exposure through use of chemical for household cleaning, use of mosquito repellents and through dietary routes.

As many as 21 pesticides belonging to all the different categories, *viz.:* fungicides such as Thiram and Ziram, herbicides such as Atrazine, insecticides, organochlorine pesticide such as Endosulfan α, organophosphorus pesticides, polyaromatic hydrocarbons as well as synthetic pyrothroids– Cypermethrin, Deltamethrin, etc. were detected in larger number of placental samples of the subjects in comparison to maternal and cord blood. Although the pesticides in blood are rapidly metabolized and degraded, as many as 20 pesticides were detected in more samples of maternal blood in comparison to placenta and cord blood. These included propaconazole, glyphosate, fenzaquin, δ BHC, DDT, phorate, naphalene and fenvalrate, etc. Normally, placenta acts as a barrier and prevents transmission of harmful compounds from mother to foetus. Still a few pesticides such as endosulfan β, metribuzine, imidacloprid, Acephate, etc. were preferentially detected in samples of cord blood in comparison to placenta and mother's blood.

While a large number of pesticides such as Ethion, Phosphamidion, Profenophos, Quinalphos, Delta BHC, Endrin, Heptachlor, Cypermethrin, Naphthalene, Atrazine, Metribuzine, Tebuconazole, Imidacloprid, etc. were highly quantified in Dibrugarh subjects, an equally larger number of pesticides, *viz.:* Acephate, Chlorpyriphos, Dimethoate, Malathion, Parathion, Phorate, Chlordane, Endosulfan, Deltamethrin, Pyrene, Hexaconazole, Deet, Fenzaquin, etc. were detected in Delhi subjects. Although largest number pesticides were detected in placenta of urban housewives from Delhi in comparison to TGW, AW and NTGW from Dibrugarh region, the average concentration of pesticides was higher in TGW, AW and NTGW in comparison to urban housewives from Delhi.

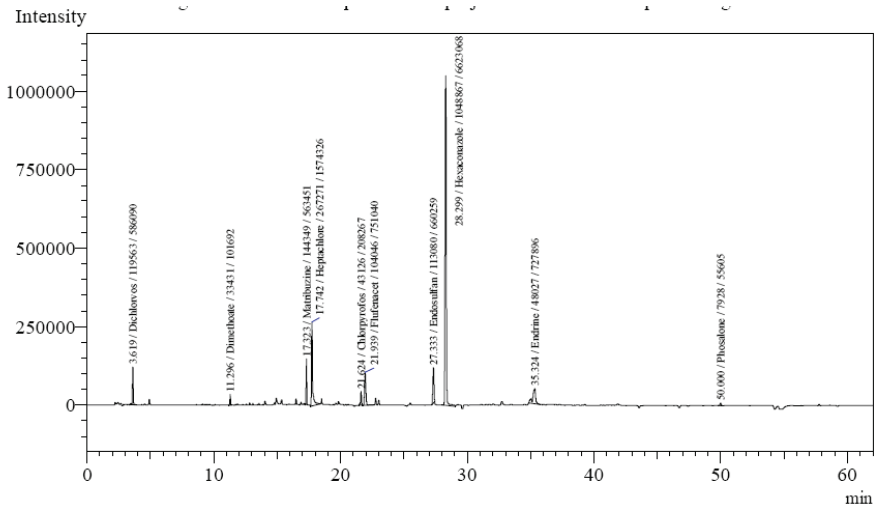
Food contaminated with endosulfan residue is the main source of animal and human exposure. Although its use has been banned in more than 60 countries owing to its high toxicity, it remains in use in several other countries, like Argentina, India and China. A high concentration of endosulfan has been observed in maternal blood of TGW. Significantly high levels of one of the most toxic compounds, alpha-endosulfan and Endosulfan sulphate (their transformation product) was found in placental tissue of NTGW and its beta isomer in cord blood of AW of Dibrugarh. Organochlorine pesticides are the most lipophilic pesticides in nature and have long half lives of months, or even years, they tend to accumulate in the adipose tissues and then are biomagnified through the food chain, thus creating a persistent exposure risk to humans. Dicofol is a pesticide that is used world-wide for agricultural applications. Since dicofol has a similar structure to DDT (dichlorodiphenyltrichloroethane), it is associated with similar concerns to DDT and its metabolites. In tea garden workers, more than 2 ppm of dicofol was observed in cord blood and maternal blood of agricultural workers.

Ethion is an organophosphate pesticide that causes cholinesterase inhibition in humans. Its lethal oral dose in humans is estimated to be 50-500 mg/kg. Acute oral exposure causes severe gastrointestinal effects, such as cramps, diarrhoea, nausea, and anorexia. Very high concentration of ethion was observed in placenta of tea-garden workers. Similarly high concentrations of Cypermethrin, one of major ingredients of mosquito-repellent, DDT – a banned organochlorine pesticide and dimethoate have been observed in placental tissue of AW of Dibrugarh. Malathion is a widely used insecticide and acaricide used for the control of aphids, red spider mites, leaf hoppers and thrips on a wide range of vegetable and other crops. It is also used to control insect vectors like mosquitoes. We have observed a significantly high concentration of malathion in housewives of Delhi and in placental tissue of agriculture workers. More than 2 ppm of Chlorpyrifos, fourth highest consumed pesticide in India was detected in Delhi samples. It is a widely applied insecticide in homes and restaurants against cockroaches and termites. Endrin, a chlorinated hydrocarbon insecticide, produces hyper excitability of the human central nervous system. High concentrations of endrin were observed in placental tissue of TGW, AW as well as NTGW of north-east.



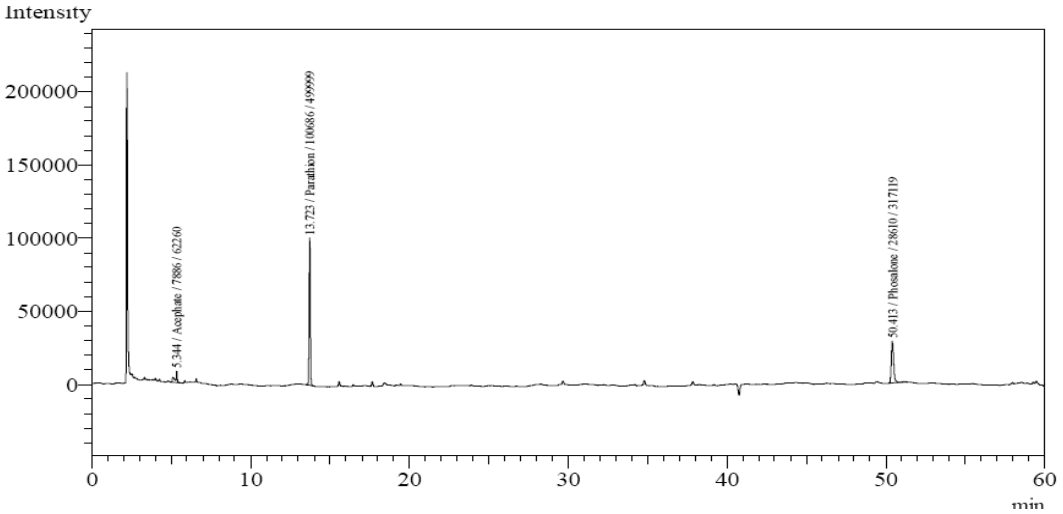
Peak#	Ret.Time	Area	Height	Units	Name
1	12.302	23696	4882	ppm	DeltaBHC
2	17.304	5689229	1025930	ppm	Heptachlore
3	20.979	1062777	124331	ppm	Malathion
4	45.086	1527989	145525	ppm	Dicofole
5	50.540	275921	25689	ppm	Phosalone
6	61.532	280317	52977	ppm	Cypermethrine
Total		8859929	1379334		

Fig 3: Chromatogram of placental extract of tea-garden worker



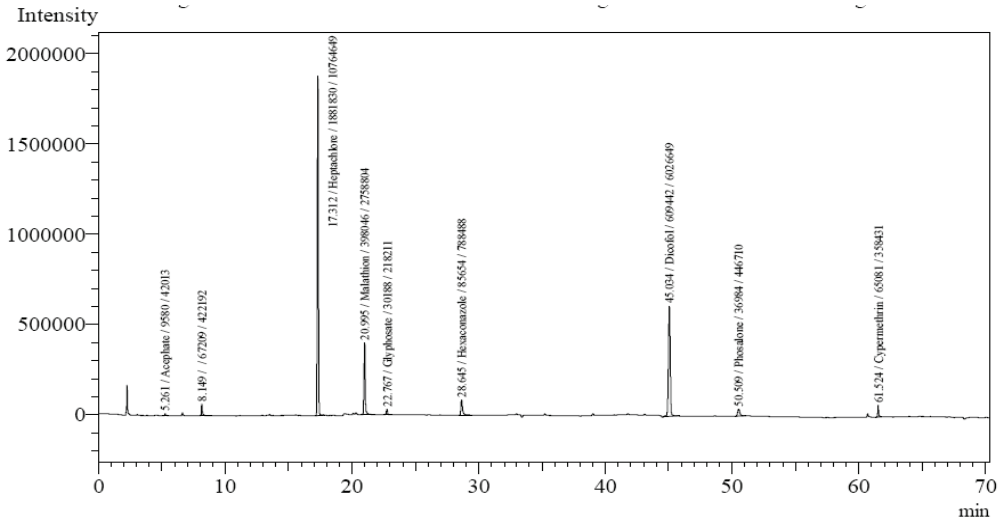
Peak#	Ret.Time	Area	Height	Units	Name	Peak Start	Area%	Area/Height	Resolution
1	3.619	586090	119563	ppm	Dichlorvos	3.333	4.9452	4.902	0.000
2	11.296	101692	33431	ppm	Dimethoate	11.196	0.8580	3.042	77.785
3	17.323	563451	144349	ppm	Matribuzine	17.141	4.7542	3.903	69.651
4	17.742	1574326	267271	ppm	Heptachlore	17.645	13.2836	5.890	3.779
5	21.624	208267	43126	ppm	Chlorpyrofos	21.430	1.7573	4.829	32.604
6	21.939	751040	104046	ppm	Flufenacet	21.766	6.3370	7.218	1.991
7	27.333	660259	113080	ppm	Endosulfan	27.198	5.5710	5.839	30.458
8	28.299	6623068	1048867	ppm	Hexaconazole	28.001	55.8829	6.314	6.075
9	35.324	727896	48027	ppm	Endrine	34.856	6.1417	15.156	27.947
10	50.000	55605	7928	ppm	Phosalone	49.808	0.4692	7.014	55.856
Total		11851694	1929688				100.0000		

Fig. 4: Chromatogram of maternal blood of tea-garden worker



Ret.Time	Area	Height	Units	Name	Peak Start	Area%	Area/Height	Resolution
5.344	62260	7886	ppm	Acephate	4.975	7.0800	7.895	0.000
13.723	499999	100686	ppm	Parathion	13.492	56.8583	4.966	77.905
50.413	317119	28610	ppm	Phosalone	50.237	36.0617	11.084	178.633
	879378	137182				100.0000		

Fig. 5: Chromatogram of maternal blood of urban housewife.



Peak#	Ret.Time	Area	Height	Units	Name
1	5.261	42013	9580	ppm	Acephate
2	8.149	422192	67209		
3	17.312	10764649	1881830	ppm	Heptachlore
4	20.995	2758804	398046	ppm	Malathion
5	22.767	218211	30188	ppm	Glyphosate
6	28.645	788488	85654	ppm	Hexaconazole
7	45.034	6026649	609442	ppm	Dicofol
8	50.509	446710	36984	ppm	Phosalone
9	61.524	358431	65081	ppm	Cypermethrin
Total		21826147	3184014		

Fig. 6: Chromatogram of placental extract of agriculture worker

2. DYNAMICS OF ULTRA-STRUCTURAL AND IMMUNOLOGICAL EVENTS IN RESPONSE TO TREATMENT IN DIFFERENT FORMS OF PSORIASIS: CO-MORBIDITIES ASSOCIATED WITH PSORIASIS

Scientific Staff	: Dr. Arun Kumar Jain, Dr. Usha Agrawal, Dr. Avninder Pal Singh
In collaboration with	: Dr. Ridhi Arya, Dr. Poonam Puri, Dr. V Ramesh, SJH, New Delhi
Technical Staff	: Mrs. Asha Rani Srivastava, Mrs. Anita Bhatia, Mr. Suresh
Duration	: 2010-12

Aims, Objectives & Background

Psoriasis is a common and chronic inflammatory skin disease, which is characterized by an extremely increased rate of epidermal turnover, and an activated mononuclear infiltrate in the underlying dermis (Fine 1988; Arican 2005). It affects up to 2.5% of the world's populations (Safer 2002) and comprises of red, scaly patches on skin, which usually have very well defined edge and appear covered by silvery flaky surface. The pathogenesis of psoriasis involves a complexity of physiological, immunological, environmental and genetic factors and the exact molecular pathogenic mechanism of psoriasis is largely unknown. Several factors such as injury to the skin, vaccinations and certain medications are reported to trigger or are responsible for the development of this condition. Excessive alcohol consumption, obesity, lack of or overexposure to sunlight, stress, cold climate and general poor health result in flaring up of psoriasis.

Psoriasis has been reported to be associated with serious co-morbidities. Emerging co-morbidities of psoriasis include cardiovascular disease, metabolic syndrome, hypertension, dyslipidemia, non-alcoholic fatty liver disease, hyperthyroidism and insulin resistance. The relationship between psoriasis and co-morbidities such as metabolic syndrome and cardiovascular disease is likely linked to underlying chronic inflammatory nature of psoriasis. Certain endocrinological disturbances are assumed to exacerbate psoriasis. Several studies have reported significant changes in the level of various thyroid

hormones in psoriasis, however, there is no consensus with regards to the extent of these variations. While one study reported significantly elevated serum TT4 (total T4) and serum free T3 (FT3) levels, other groups observed significantly elevated levels of FT4 in the patient group. It has been reported that when thyroid function is low, prolactin increases. Goswami et al (2009) reported positive correlation between serum TSH and prolactin levels. Prolactin is an important immunomodulator and exerts a proliferative effect in cultured human keratinocytes via specific receptors.

The objectives of this study are to find occurrence of co-morbities, *viz.:* hyperthyroidism, Non-Alcoholic Fatty Liver Disease (NAFLD), psoriatic arthritis, dyslipidemia and hypertension in patients of psoriasis and to assess the incidence of metabolic syndrome in psoriasis patients.

Work done during the year

During the year under report, 65 patients with clinical presentation of psoriasis and 40 controls were enrolled for the study. Patients receiving systemic treatment for psoriasis including acitretin, cyclosporin, methotrexate, phototherapy in the preceding four weeks at presentation were excluded from the study. Attempts were made to record history pertaining to hypothyroidism, nephrotic syndrome, obstructive liver disease, connective tissue disease, to rule out secondary causes of hyper-lipidemia. Patients were examined clinically for type of lesion, number of lesions and extent of severity of the disease which is assessed by Psoriasis Area Severity Index Score (PASI Score).

Blood samples were collected from the psoriatic patients as well as the control group for estimation of different thyroid hormones and lipid profile. Serum was separated and levels of free T3 (FT3), total T4 (TT4), and TSH were estimated by ELISA (Enzyme-Linked Immunosobent Assay). The levels of serum Total Cholesterol (TC), HDL-C, LDL-C and Triglycerides (TG) were determined by enzymatic methods using a Roche Hitachi 902 Autoanalyzer (*Randox*).

The study revealed comparatively higher levels of LDL cholesterol in psoriatic patients ranging from 76-167 mg/dL (mean- 114±26) in comparison to 64-190 mg/dL in control group (mean- 102±28). Sixteen psoriatic patients had normal LDL level while 5 patients had high level of LDL (Table 1). In comparison, only 1 out of 18 controls showed higher

than normal range of LDL cholesterol. Majority of psoriatic patients (12 out of 21) had low HDL level which ranged from 25-82 mg/dL (mean 40 ±13). On the other hand, in the control group, 9 subjects had normal HDL level. Cholesterol level in psoriatic patients ranged from 106-212 mg/dL (mean 159±28), in comparison to 60-213 mg/dL (mean 128±47.1) in the control group. The triglycerides level in psoriatic patients ranged from 76-322 mg/dL (mean133±54.3) with 4 patients exhibiting high level than reference range. On the other hand, in the control group, the triglyceride levels ranged from 60-213 mg/dL (mean122±47.1).

Table 1: Lipid profile in psoriasis patients and control group

	Patients (n=21)	Controls (n=18)
LDL	114±26 (76 to 167)	102±28 (64 to 190)
HDL	40±13 (24 to 65)	40±13 (25 to 82)
Cholesterol	159±28 (106 to 212)	160±50 (26 to 293)
Trglycerides	133±54.3 (76 to 322)	128±47.3 (60 to 213)
Cholesterol/HDL	5±1 (2 to 7)	5±1 (3 to 8)

The ratio of cholesterol to HDL level in psoriatic patients ranged from 2 - 7 (mean 5±1). In comparison the control group showed Cholesterol: HDL ratio from 3 - 8 (mean 5±1).

The level of free thyroid hormone (free T3) ranged from 0.005 - 0.417 ng/mL (mean 0.132±0.136) in psoriatic patients, while in control group it ranged from 0.005 - 0.488 ng/mL (mean 0.112±0.123).

Table 2: Level of thyroid hormones in psoriasis patients and control group

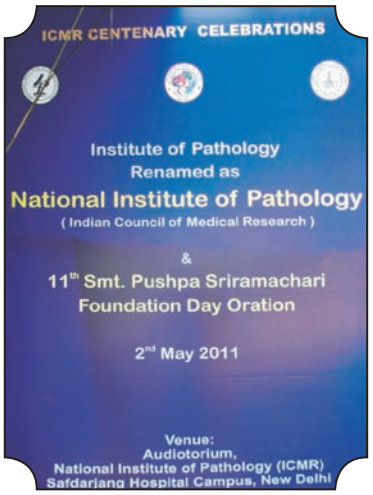
Thyroid Hormones	Psoriatic patients (n=22) Mean ± SD (Range)	Control group (n=20) Mean ± SD (Range)
FT3	0.132±0.136 (0.005 to 0.417)	0.112±0.123 (0.005 to0.488)
TT4	3.7±0.85 (2.65 to3.75)	3.6±0.51 (2.71 to4.41)
TSH	4.44±1.36 (2.17 to 7.04)	4.05±1.33 (1.97 to 7.34)

As shown in Table 2, the level of T4 in psoriasis ranged from 2.65 - 5.75 µg/dL (mean 3.7±0.85) in comparison to 3.6 ± 0.51 (range 2.71 - 4.41 µg/dL) observed in the control group. Further, the TSH level in psoriatic patients ranged from 2.17 - 7.04 µIU/mL (mean 4.44±1.36), while in control patients it ranged between 1.97 - 7.34 µIU/mL (mean4.05±1.33). The study is in progress.


ACADEMIC
ACTIVITIES

MAJOR ACTIVITIES AT NATIONAL INSTITUTE OF PATHOLOGY

1. Institute attained the status of **'National Institute of Pathology'** on the Foundation Day of Institute of Pathology on 2nd May 2011.

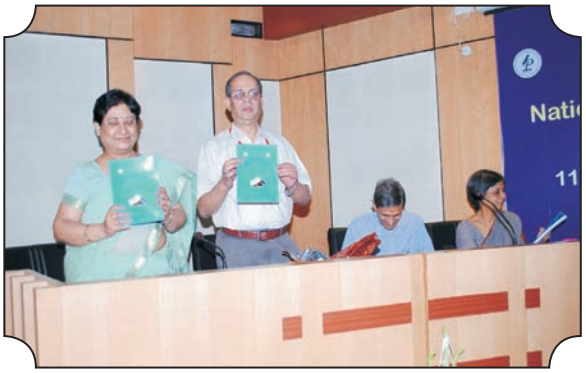


2. Annual meeting of **IAPM (Delhi Chapter)** was organized by National Institute of Pathology (ICMR) with Department of Pathology, Safdarjang hospital at Vallabhbhai Patel Chest Institute on 17th April 2011.

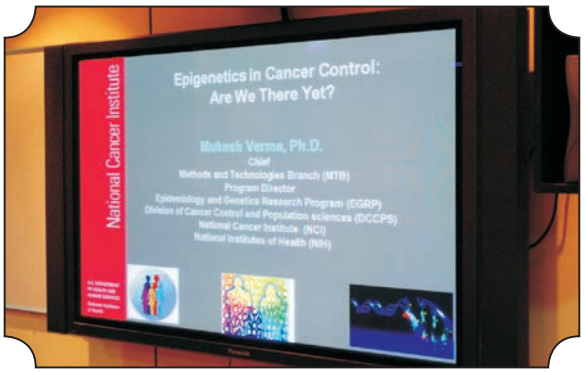
3. Organized **Director's Meeting of Indian Council of Medical Research** from 8th-9th May 2011.

4. Organized **'Foundation Workshop on Clinical and Laboratory Medicine Research'** with Moving Academy of Medicine and Biomedicine, Pune on 26th-28th July 2011.

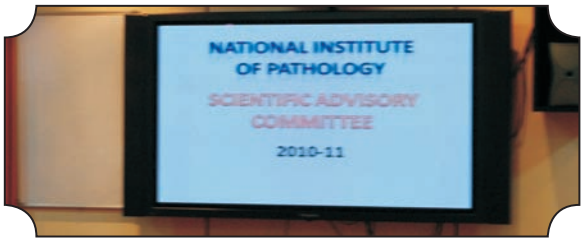
5. **11th Smt. Pushpa Sriramachari Foundation Day Oration** was delivered by **Prof. Avdesh Surolia** on ***'Harnessing protein folding to counter diabetes and other diseases'*** at National Institute of Pathology (ICMR) on 2nd May 2011.



6. **Professor Mukesh Verma**, Chief, Methods and Technology Branch, Programme Director, Epidemiology and Genetics Research program, National Cancer Institute (NCI), Division of Cancer Control and Population Sciences, National Institutes of Health (NIH), USA delivered a lecture on ***'Epigenetic in Cancer Control: Are we there yet?'*** at National Institute of Pathology (ICMR) on 19th December 2011.

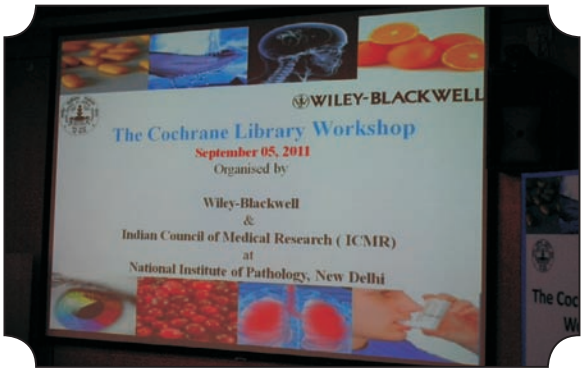


7. **Scientific Advisory Committee (SAC)** for the year 2010-11 of National Institute of Pathology (ICMR) was held on 22nd November 2011. Students presented posters on their work for review by experts.



8. Workshop on **'Flow Cytometry**: **Technical Support and Education (TTE) Programme'** was organized by National Institute of Pathology (ICMR) and BD Biosciences at National Institute of Pathology from 15th-17th February 2012.

9. Organized one-day **'Library Training Workshop'** sponsored by ICMR and Wiley Publications on **'Cochrane Library'** at National Institute of Pathology (ICMR) on 5th September 2011.



10. On the eve of **15th August 2011** and **26th January 2012**, flag hoisting was done at National Institute of Pathology (ICMR).

11. **Post-graduate students from Government Medical College, Amritsar** visited different laboratories of the Institute to get exposure to high throughput technologies available at National Institute of Pathology (ICMR).
12. Inspection of National Institute of Pathology by **Dr. Rajiv Sen**, PGIMS, Rohtak, for **renewal of accreditation of DNB Program by NBE** on 20th July 2011.

13. **Dr. Hari S. Sharma,** Institute of Cardiovascular Research, YUMC, University Medical Centre, Amsterdam, The Netherlands, delivered guest lecture on '***Molecular Patho-physiology of chronic lung diseases'*** on 27th September 2011.
14. **Dr. Thierrry Dameral**, Deputy CEO Inserm and **Dr. Philippe Arthets**, Deputy Director, External Relations, Inserm, France, visited National Institute of Pathology (ICMR) on 19th October 2011.
15. Organized **Vigilance Awareness Week** from 31st October 2011 to 5th November 2011 at National Institute of Pathology (ICMR).

16. **World Health Organization (WHO)-sponsored 'Training Course for Technicians** and **Advanced level** held at National Institute of Pathology (ICMR) from 24th November 2011 uptill 23rd December 2011 on the topic, ***'Basic Biostatistics and Epidemiology'***.
17. Organized one-day workshop on **'Application of Next Generating Sequencing (NSG) in Medical Research'**, held jointly by National Institute of Pathology and Indian Council of Medical Research at National Institute of Pathology on 28th February 2012.
18. National Institute of Pathology (ICMR) organized workshop on ***'Application of Molecular Biology in Cancer Diagnostics'*** at Dr. B. Barooah Cancer Institute jointly with BBCI, Guwahati on 28th February-1st March 2012.

AWARDS / HONOURS

1. **Dr. Sujala Kapur** awarded **'Membership of National Academy of Medical Sciences'**.

2. **Dr. Poonam Salotra** was elected as fellow of Indian National Science Academy (INSA fellow) w.e.f Jan. 2012.

3. **Dr. Poonam Salotra** served as a member of WHO Expert committee on Leishmaniasis.

4. **Dr. Ruchi Singh** was awarded with the best presentation award in women scientist category in the National Symposium on Microbes in Health and Agriculture, organized by School of Life Sciences, Jawaharlal Nehru University, New Delhi from 12th-13th March 2012.

5. **Dr. Fauzia Siraj** got '**Ramalingaswamy Award'** for the best paper on "Immunohistochemical expression of chemokine receptor CXCR3 and its ligand CXCL10 iastrocytomasin" in annual meeting of IAPM – Delhi chapter.

EXTRAMURAL PROJECTS (2011-12)

NEW PROJECTS

1. **Development of new live attenuated vaccine candidates for kala-azar.**
   Dr. Poonam Salotra- DBT (2011-14).

2. **Study on effects of anti-cancer drugs on breast cancer stem cells.**
   Dr. Sunita Saxena- ICMR (2011-13).

3. **Epigenetic studies in esophageal cancer in high risk region of north-east India.**
   Dr. Sunita Saxena, Dr. Sujala Kapur- Twining Program of DBT (2011-14).

ONGOING PROJECTS

1. **Genome wide analysis of genetic alterations in patients with esophageal cancer from north-east India using single nucleotide polymorphism array.**
   Dr. Sujala Kapur, Dr. Sunita Saxena- ICMR (2011-14).

2. **Study on the effect of siRNA mediated androgen receptor gene silencing on androgen signaling pathway in breast carcinoma.**
   Dr. Anurupa Chakraborty- Post-Doctoral Fellowship, ICMR (2010-12).

3. **Clinicopathological determinants in leprosy type 1 reactions.**
   Dr. Avninder Pal Singh- ICMR Leprosy Task Force project (2010-12).

4. **Immunogenetic profile of nasopharyngeal cancer.**
   Dr. Sujala Kapur, Dr. Sunita Saxena- DBT (2010-13).

5. **Characterization of host immune factors associated with progression of superficial TCC of bladder by microarray analysis.**
   Dr. Sunita Saxena, Dr. Usha Agrawal- ICMR (2009-12).

6. **Study on gene expression and hypermethylation profiles in early onset breast cancer.**
Dr. Sunita Saxena, Dr. Sujala Kapur, Dr. BSA Raju- DBT (2008-11).

7. **Protective immunogenicity of centrin KO live attenuated *Leishmania* parasite in the animal models and in the human cells.**
Dr. Poonam Salotra- DBT (2011-13).

8. **New tools for monitoring drug resistance and treatment response in visceral leishmaniasis in the Indian subcontinent.**
Dr. Poonam Salotra- European Commission (2009-13).

9. **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 (2009-12).

10. **Transcriptome profiling for identification and characterization of miltefosine resistance associated genes of *Leishmania donovani*.**
Dr. Poonam Salotra- ICMR (2009-12).

11. **Detection of *Chlamydia trachomatis* in synovial samples from patients with undifferentiated spondyloarthropathy / reactive arthritis.**
Dr. Sangita Rastogi- SRF, ICMR (2010-13).

12. **A novel arithmetic approach for fool-proof production of growth arrest in 3T3 cells suitable for human epidermal culture.**
Dr. LK Yerneni- ICMR (2010-13).

13. **Studies on epithelial-mesenchymal interactions using human epidermal keratinocyte stem cells and innovatively growth-arrested fibroblast feeders in 3-D collagen model.**
Dr. LK Yerneni- SRF, ICMR (2010-13).

COMPLETED PROJECTS

1. Establishment of breast cancer cell lines from primary breast tumours.
   Dr. Sunita Saxena, Dr. Sujala Kapur, Dr. BSA Raju- ICMR Multicentric Task Force Project (2007-11).

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- DST (2008-11).

3. Assessment of pesticide exposure in tea garden workers of north-eastern state of India (HEBM).
   Dr. AK Jain- ICMR (2008-11).

PUBLISHED PAPERS

1. Ihsan R, Chauhan PS, Mishra AK, Yadav DS, Kaushal M, Sharma JD, Zomawia E, Verma Y, Kapur S, Saxena S. Multiple analytical approaches reveal distinct gene-environment interactions in smokers and non-smokers in lung cancer. **PLoS One**, 6(12): e29431, 2011.

2. Chauhan PS, Bhushan B, Singh LC, Mishra AK, Saluja S, Mittal V, Gupta DK, Kapur S. Expression of genes related to multiple drug resistance and apoptosis in acute leukemia: Response to induction chemotherapy. **Exp Mol Pathol**, 92(1):44-49, 2011.

3. Chauhan PS, Bhushan B, Mishra AK, Singh LC, Saluja S, Verma S, Gupta DK, Mittal V, Chaudhry S, Kapur S. Mutation of FLT3 gene in acute myeloid leukemia with normal cytogenetics and its association with clinical and immunophenotypic features. **Med Oncol**, 28(2): 544-551, 2011.

4. Agarwal Usha, Mishra AK, Salgia Payal, Verma Saurabh, Mohanty NK, Saxena S. Role of tumour suppressor and angiogenesis markers in prediction of recurrence of non-muscle invasive bladder cancer. **Pathol and Oncol Res**, 17(1): 91-100, 2011.

5. Bansal A, Bhatnagar A, Saxena S. Metastasizing granular cell ameloblastoma**. J Oral Maxillofac Pathol**, 16: 122-124, 2012.

6. Singh LC, Chakraborty A, Kapur S, Saxena S. Study on predictive role of AR and EGFR family genes with response to neo-adjuvant chemotherpy in locally advanced breast cancer in Indian women. **Med Oncol**, 29: 539-546, 2012.

7. Singh A, Mishra AK, Ylaya K, Hewitt SM, Sharma KC, Saxena S. Wilms Tumor-1, Claudin-1 and Ezrin are useful immunohistochemical markers that help to distinguish schwannoma from fibroblastic meningioma. **Pathol Oncol Res**, DOI 10.1007/s12253-011-9456, 2011.

8. Kohli S, Singh Y, Sharma K, Mittal A, Ehtesham NZ, Hasnain SE. Comparative genomic and proteomic analyses of PE/PPE multigene family of Mycobacterium tuberculosis H37Rv and H37Ra reveal novel and interesting differences with implications in virulence. **Nucleic Acids Res,** 40:7113-22, 2012.

9. Tyagi A, Kumar U, Reddy S, Santosh VS, Mohammed SB, Ehtesham NZ, Ibrahim A)

Attenuation of colonic inflammation by partial replacement of dietary linoleic acid with α-linolenic acid in a rat model of inflammatory bowel disease. **Brit. J. Nutr.,** 16: 1-11, 2011.

10. Suragani M, Rasheedi S, Hasnain SE, Ehtesham NZ. The translation initiation factor, PeIF5B, from Pisum sativum displays chaperone activity. **Biochem Biophys Res Commun.,** 414:390-6, 2011.

11. Alvi A, Ansari SA, Ehtesham NZ, Rizwan M, Devi S, Sechi LA, Qureshi IA, Hasnain SE, Ahmed N.) Concurrent proinflammatory and apoptotic activity of a Helicobacter pylori protein (HP986) points to its role in chronic persistence. **PLoS One**, 6:e22530. 46**,** 2011.

12. Ehtesham NZ, Nasiruddin M, Alvi A, Kumar BK, Ahmed N, Peri S, Murthy KJ, Hasnain SE.) Treatment end point determinants for pulmonary tuberculosis: human resistin as a surrogate biomarker. **Tuberculosis,** 91:293-9**,** 2011.

13. Hasnain SE, Banerjee S, Ehtesham NZ. Translating advances in genomic research into clinical practice: the challenges ahead. **Med Princ. Pract.,** 20:392-4**,** 2011.

14. Banerjee S, Farhana A, Ehtesham NZ, Hasnain SE. Iron acquisition, assimilation and regulation in mycobacteria. **Infect. Genet. Evol.,** 11:825-38, 2011.

15. Katara GK, Ansari NA, Verma S, Ramesh V, Salotra P. Foxp3 and IL-10 expression correlates with parasite burden in lesional tissues of post kala-azar dermal leishmaniasis (PKDL) patients. **PLoS Negl Trop Dis,** 5(5): e1171, 2011.

16. Khandelwal K, Bumb RA, Mehta RD, Kaushal H, Lezama-Davila C, Salotra P, Satoskar AR. A patient presenting with diffuse cutaneous leishmaniasis (DCL) as a first indicator of HIV infection in India. **Am J Trop Med Hyg**, 85(1): 64-65, 2011.

17. Kulshrestha A, Singh R, Kumar D, Negi NS, Salotra P. Antimony-resistant clinical isolates of *Leishmania donovani* are susceptible to paromomycin and sitamaquine. **Antimicrob Agents Chemother,** 55(6): 2916-2921, 2011.

18. Sreenivas G, Sharma P, Duncan R, **Salotra P**, Nakhasi HL. Mitochondrial associated Ubiquitin fold modifier-1 mediated protein conjugation in *Leishmania donovani*. **PLoS One;** 6 (1):e16156 (2011).

19. Prasad N, Ghiya B, Kaushal H, Satoskar AA, Davila Claudia L, Salotra P, Bumb RA, Satoskar AR. Heat, Oriental Sore, & HIV. The Lancet, 377:610 (2011)

20. Ramesh V, Katara GK, Verma S, Salotra P. Miltefosine as an effective choice in the treatment of post-kala-azar dermal leishmaniasis. **Br J Dermatol**, 165(2): 411-414, 2011.

21. Selvapandiyan A, Dey R, Gannavaram S, Lakhal-Naouar I, Duncan R, Salotra P, Nakhasi HL. Immunity to visceral leishmaniasis using genetically defined live-attenuated parasites. **J Trop Med**, 631460, 2012.

22. Soni P, Prasad N, Khandelwal K, Ghiya BC, Mehta RD, Bumb RA, Salotra P. Unresponsive cutaneous leishmaniasis and HIV co-infection: Report of three cases. **Indian J Dermatol Venereol Leprol**, 77(2): 251, 2011.

23. Srividya G, Kulshrestha A, Singh R, Salotra P. Diagnosis of visceral leishmaniasis: Developments over the last decade. **Parasitol Res**, 110(3): 1065-1078, 2012.

24. Rath G, Roy S, Goyal U, Jain AK and Mukherjee A. Placental vasculosyncytial membrane in tobacco exposed Indian mothers: A quantitative and ultrastructural study. J**ournal Internat. Med. Sci. Acad.,** 24(2): 53-56 (2011)

25. Soni S, Rath G, Prasad C. P, Salhan S, AK Jain and Saxena S. Fas-FasL System in Molar Pregnancy. **Am J Reprod Immunol.,** 65(5): 512-520, 2011.

26. Kumar SN, Telang AG, Singh KP, Jain AK, Afroz M and Patil RD. Experimentally induced toxicity of Ochratoxin A and Endosulfan in male wistar rats: A hormonal disorder. **Journal of Animal and Veterinary Advances,** 10(13): 1750-1755, 2011.

27. Rath G, Soni S,. Prasad C. P, Salhan S, Jain AK and Saxena S. Bcl-2 and p53 Expression in Indian Women with Complete Hydatidiform Moles. **Singapore Medical Journal,** 52(7): 502-507, 2011.

28. Rao RP, Jain AK and Srinivasan BP, Dual therapy versus monotherapy of trandolapril and telmisartan on diabetic nephropathy in experimentally induced type 2 diabetes mellitus rats. **Journal of the Renin-Angiotensin-Aldosterone System,** 12(3): 169-175, 2011.

29. Rao RP, Singh A, Jain AK and Srinivasan BP, Dual therapy of rosiglitazone/ pioglitazone with glimepiride on diabetic nephropathy in experimentally induced type 2 diabetes rats. **Journal of Biomedical Research,** 25(6): 411-417, 2011.

30. Bihaqi SW, Singh AP, Tiwari M. *In-vivo* investigation of the neuroprotective property of *Convolvulus pluraicaulis* in scopolamine-induced cognitive impairments in Wistar rats. **Indian J Pharamacol,** 43: 520-525, 2011.

31. Ramesh V, Singh A, Pahwa M, Capoor M. A recalcitrant facial plaque: Fixed cutaneous sporotrichosis. **Int J Dermatol,** 50: 367-368, 2011.

32. Capoor MR, Ramesh V, Khanna G**,** Singh A, Aggarwal P. Sporotrichosis in Delhi among migrant population from Uttarakhand, India. **Trop Doct,** 41: 46-48, 2011.

33. Malhotra P, Arora D, Singh A. Squamous cell carcinoma, syringocystedenoma papilliferum and apocrine adenoma arising in a nevus sebaceous of Jadssohn. **Indian J Pathol Microbiol,** 54: 225-226, 2011.

34. Jairajpuri Z, Jain I, Singh A. Papillary meningioma: A rare malignant variant. **Indian J Pathol Microbiol,** 54: 410-411, 2011.

35. Puri P, Singh A, Ramesh V. Facial nodule in a 34-year-old man. **Indian J Dermatol,** 56: 230-231, 2011**.**

36. Malhotra A, Singh A, Ramesh V. Basal cell carcinoma in the north Indian population: Clinicopathologic review and immunohistochemical analysis. **Ind J Dermatol Venreol Leprol,** 77(3): 328-330, 2011.

37. Mondal Shankar, Varma Saurabh, Bamola Vishwa Deepak, Naik Satya Narain, Mirdha Bijay Ranjan, Padhi Madan Mohan, Mehta Nalin, Mahapatra Sushil Chandra. Double-blinded randomized controlled trial for immunomodulatory effects of Tulsi (*Ocimum sanctum Linn*) leaf extract on healthy volunteers. **Journal of Ethanopharmacology**, 136: 452-456, 2011.

ACCEPTED FOR PUBLICATION

1. Nyati KK, Prasad KN, Kharwar NK, Soni P, Husain N, Agrawal V and Jain AK. Immunopathology and Th1/Th2 immune response of Campylobacter jejuni-induced paralysis resembling Guillain–Barre' syndrome in chicken. Med Microbiol Immunol.

2. Soni A, Bansal A, Mishra AK, Batra J, Singh LC, Chakraborty A, Yadav DS, Mohanty NK, Saxena S. Functional Association of androgen signaling (AR, PSA) and metabolizing (CYP19) gene polymorphisms in prostate carcinoma in north Indian population. Genetic Testing and Molecular Biomarker.

3. Mishra AK, Agrawal U, Negi S, Bansal A, Bhatnagar A, Bhatnagar D, Chintamani, Mohil R, Saxena S. Study on expression of AR in breast cancer and its correlation with other steroid receptors and growth factors. Indian Journal of Medical Research, 2011.

4. Rastogi S, Sharma VK, Khanna G, Batra S, Kumar P. Detection of *Chlamydia trachomatis* in synovial fluid of patients with reactive arthritis. J Clin Rheum.

5. Prasad P, Singh N, Das B, Rastogi S. Involvement of superoxide dismutase in pathogenesis of spontaneous abortion in women infected with *Chlamydia trachomatis*. J Obstet Gynecol.

6. Bansal Anju, Soni Abha, Rao Punita, Singh LC, Mishra Ashwini, Mohanty N K, Saxena Sunita. Implication of DNA repairs genes in prostate carcinogenesis in Indian men. Indian Journal of Medical Research (In Press).

BOOKS / MONOGRAPHS

1. Mishra AK, Chakraborty A, Saxena S. Significance of vitamin D receptor polymorphisms in breast cancer- Multinomial logistic regression analysis. In: Vitamin D: Nutrition, Side Effects & Supplements. Ed.: Malone Stephanie R.

2. Saxena Sunita. Molecular and genetic aspects of lung cancer. chapter 13, pp. 141-148. In: Pathological & Occupational Lung Health. Eds.: Vijyan VK, Tazelaar HK, Kulshrestha Ritu.

3. Varma S. Tissue Transglutaminase (tTG) expression in human brain tumours: An immunohistochemical study. Lambert Academic Publications, Germany, 2011.

4. Singh A, Weng X, Nath I. Skin biopsy in leprosy. In: Skin Biopsy, InTech Publishers, 5: pp. 73-86, 2011.

5. Singh R, Kulshrestha A, Salotra P. Research in diagnostic tools: the past, present and future. In "Kala azar- Emerging perspectives and prospects in South Asia", Ed. H.P Thakur, Mittal publishers India PP 155-189 (2011).

SCIENTIFIC ACTIVITIES

Dr. Sunita Saxena

1. Chaired the session on 'Disease Proteomics (Infectious Diseases)' at the IInd Annual Indian Proteomics Conference (IPCON-2011) held in New Delhi on 3rd-5th April 2011.

2. Inspected the laboratory of Dr. Yogeshwar Shukla, E-II, ECO, IITR, Lucknow on 11th April 2011 as member of ICMR team.

3. Invited to attend the meeting to discuss the policy for storage of biological samples in ICMR-funded project on 25th April 2011 at ICMR, New Delhi.

4. Invited to attend Project Review Committee meeting for Cellular and Molecular Biology and Genomics as expert on 20th May 2011 at ICMR, New Delhi.

5. Chairperson of Ethical Committee of Safdarjung hospital, New Delhi and regularly chaired the meetings of Ethics committee on alternate months.

6. Attended meetings of the Specification Committee for installing laboratory equipments and furniture for the National Institute of Research in Environmental Health, Bhopal on 6th June and 18th October 2011 at ICMR, New Delhi.

7. Invited as member of Selection Committee for the selection of candidates for ICMR-Post Doctoral Fellowship and Research Associate held on 16th June 2011 and 22nd December 2011 respectively at ICMR, New Delhi.

8. Attended meeting on Cancer Monograph in ICMR on 19th July 2011 at ICMR, New Delhi.

9. Member of Condemnation Board and attended meetings of the Condemnation Board of ICMR held on 27th July 2011 at National Institute of Medical Statistics, New Delhi.

10. Nominated to attend WG-13: Working Group of the Department of Atomic Energy (R & D Sector) held on 1st August 2011 at Mumbai, for preparation of 12th plan.

11. Attended meeting of Scientific Advisory Committee of Institute of Cytology & Preventive Oncology, Noida on 3rd August 2011.

12. Invited as Chief Guest at National conference on 'Current Trends in Advanced Biomedical Technology (CTA B-II)' organized by Department of Biosciences, Nehru Arts and Sciences College, Coimbatore, during 20th - 21st September 2011 and delivered Key-note address on *'Understanding Molecular Biology of Cancer using Genomic Approaches'.*

13. Member of Project Review Committee in Oncology (NCD) and attended meetings on 22nd–23rd September 2011 at ICMR, New Delhi.

14. Attended workshop on 'Clinical Trials Registry– India amongst the Ethics Committee' held on 26th September 2011 at India International Centre Annex, New Delhi.

15. Attended meeting of Expert Group on 'Effectiveness and safety of 2 vs 3 doses of HPV vaccine in prevention of cervical cancer: An Indian multicentric randomized trial' held on 28th September 2011 at ICMR, New Delhi.

16. Attended 2nd meeting of the PRSG to review the project 'Development of PC-based fully automatic batch analyzer for clinical chemistry' held on 17th October 2011 at CSIO, Chandigarh organized by Department of Information Technology, New Delhi.

17. Attended Technical Committee meetings of ICMR held on 4th November 2011, 19th January & 19th March 2012 at ICMR, New Delhi.

18. Attended Selection Committee Meeting for award of Emeritus Medical Scientist for ICMR held on 4th November 2011 at ICMR, New Delhi.

19. Nominated as member of Scientific Advisory Committee of Regional Medical Research Centre, NE region, Dibrugarh (Assam).

20. Attended Scientific Advisory Group Meeting of Division of Publication & Information, ICMR held at ICMR, New Delhi on 29th November 2011.

21. Invited to deliver talk on *'Significance of Microarray Technology in Understanding Cancer Genome and identification of Biomarker– Genome-wise approach to identify Cancer Biomarker"* in 1st Biennial Conference of IASN organized by Agra Medical College in association of National JALMA Institute for Leprosy and Other Mycobacterial Diseases (ICMR), Agra during 30th November - 1st December 2011.

22. Invited to deliver talk on *'Application of Tissue Microarray in Pathology'* in Symposium on "Antibody–based Proteomics' on 1st December 2011 at IAP-APCON 2011.

23. Nominated as expert for Selection Committee meeting for NASI Scopus Young Scientist Award 2011 held on 5th December 2011 in New Delhi.

24. Attended Scientific Advisory Group meeting of Basic Medical Science Division of ICMR held on 26th December 2011 at ICMR, New Delhi.

25. Invited to attend panel discussion on 'Histopathological insight into DES thrombosis from large necropsy & experimental animal experiences: Can we have a safe DES?' organized by Department of Cardiology, VMMC & Safdarjang hospital, New Delhi on 26th December 2011.

26. Attended Director's Meeting held at Desert Medicine Research Centre, Jodhpur during 13th-14th January 2012.

27. Attended signing of Memorandum of Understanding between ICMR and Symbiosis International University, Pune on 23rd January 2012 at ICMR, New Delhi.

28. Invited to attend Meeting of South Asian Forum for Health Research (SAFHeR) during 5th – 7th February 2012.

29. Invited as Chief Guest to deliver a talk on *'Applications of Tissue Microarray in Pathology and Research'* in workshop organized by Dr. B. Borooah Cancer Institute, Guwahati held during 28th February - 1st March 2012.

Dr. Sujala Kapur

Invited Speaker

- *Molecular Diagnosis of Lymphomas and Leukemias*: 26th Annual Conference of Indian Association of Pathologists and Microbiologists, NIOP, New Delhi, April 2011.

- *Microarray and its application*. Students Exchange Progamme of GTB Hospital, Shahdara, October 2011.

- *Microarray: Principles and Methodology*: Joint Workshop on Molecular Biology, NIOP and BBCI, Guwahati, February 2012.

Workshops / Conferences / Meetings Attended

- Workshop on Cytogenetics and Automatic Slide Scanning Workstation, organized by Carl Zeiss, 5th May 2011.
- Member, Executive Committee, 26th Annual Conference of Indian Association of Pathologists and Microbiologists, NIOP, New Delhi, April 2011.
- Workshop on Next Generation Sequencing at NIN, Hyderabad from 13th-17th June 2011.
- Member, PRC meeting on Gastroenterology, NCD, ICMR.
- Visited BBCI, Guwahati and Imphal, Manipur for collaborative projects, August 2011.
- *'New Horizons in Cancer Research',* International AACR Conference, December 2011, Gurgaon.
- *'Analysis of redox mediated B-cell activation in BCLL'* at National Conference on Oxidative Stress and Its Complication in Human Health (NCOSH-2011), Karunya University, Coimbatore.

Dr. Sangita Rastogi

1. Member, Selection Committee, Department of Microbiology, VMMC & SJ hospital, New Delhi for selection of Senior Research Fellow in ICMR-funded project (August 2011).
2. Convened Institutional Animal Ethics Committee Meeting at NIP on 23rd August 2011.
3. Attended Meeting for MD Project Protocol Presentation at VMMC & SJH, New Delhi on 20th September 2011.
4. Attended ICMR Centenary Symposium & Commemorative Currency Coin Release at ICAR (Pusa), New Delhi on 15th November 2011.
5. Presented paper titled, *Circulating antibodies to Chlamydia trachomatis and heat shock proteins'* in 5th Asian Congress on Autoimmunity at Singapore on 17th-19th November 2011.

6. Participated in conference on 'Nucleic acids in disease & disorder' at Indian Institute of Technology, New Delhi on 7th-9th December 2011.

7. Reviewed STS project proposals for 'Short-Term Studentship Program' of ICMR (2011-12).

8. Attended CME on 'Diagnosis of autoimmune disorders: The continuing dilemma' at LNJP, New Delhi on 10th-11th December 2011.

9. Participated in International Symposium on Molecular Pathology at Manekshaw Centre, New Delhi on 28th-29th January 2012.

10. Participated in 'National CME in Obstetrics & Gynecology' at AIIMS, New Delhi on 24th-25th March 2012.

Dr. Poonam Salotra

1. Presentation at Woods Hole Immuno-parasitology meeting at MA, USA in April 2011.

2. Participated in the Steering Committee Meeting of *Leishmania* Kala Drug Project at Strathclyde University, Glasgow, U.K. in June 2011.

3. Invited participant in meeting to finalize the specifications of equipment for medical colleges, held at ICMR, in June 2011.

4. Participated in the Steering Committee Meeting of *Leishmania* Kala Drug Project at Berlin, Germany in August 2011.

5. Invited by Applied Biosciences for the inauguration of Technologies Torrent Personal Genome Machine Training Facility, at Gurgaon in August 2011.

6. Attended PRC meeting on Malaria, Filariasis & Leishmaniasis held at ICMR, New Delhi in September & November 2011.

7. Organized International Project Review Meeting of European Commission-funded project (Rapsodi) partners from France, Spain, Peru, Tunisia in New Delhi, 26th-28th September 2011.

8. Invited speaker at 'Advanced WHO-IRTC/UNIL Course on Immunology, Vaccinology and Biotechnology applied to Leishmaniasis' at Lausanne, Switzerland in October 2011.

9. Participated in ICMR Awards Presentation Ceremony held at DRDO Bhawan, New Delhi, in November 2011.

10. Participated in ICMR Centenary Symposium, Commemorative Currency Coin Release held at NASC Complex, ICAR, Pusa Road, New Delhi in November 2011.

11. Participated in International Symposium Vaccines: From Discovery to Translation held at Surajkund, Delhi, November 2011.

12. Participated in INSA Fellow Awards in December 2011 at Tezpur, Assam.

13. Invited speaker in meeting on 'Monitoring of clinical outcomes of Kala-azar in the health & surveillance of drug resistance' held at Surajkund, January 2012.

14. Participated in the Steering Committee Meeting of *Leishmania* Rapsodi Project at Toulon, France during 6^{th}-12^{th} February 2012.

15. Organized Training Program on Flow Cytometry in February 2012 at NIP, New Delhi.

16. Invited speaker in Meeting on 'Diagnostics of Public Health Importance in India' held in February 2012 at National Institute of Immunology.

17. Participated in second Brain Storming Meeting of Vector Science Forum on Japanese Encephalitis and Visceral and Cutaneous Leishmaniasis, held at NIP during February 2012.

18. Participated in Kala-Azar Alleviation Initiative: 1^{st} Annual meeting held at Maples Hotel, New Delhi, March 2012.

19. Reviewed several manuscripts as the Associate Editor of BMC Infectious Diseases. Invited reviewer for manuscripts submitted to PloS ONE, PloS Neglected Tropical Diseases, Journal of Investigative Dermatology, Journal of Antimicrobial Chemotherapy, International Journal of Parasitology etc.

20. Examiner and Co-ordinator for Pre-PhD course work and PhD qualifying examination at National Institute of pathology for BITS, Pilani programme.

21. Co-ordinator of weekly Journal Club at NIP.

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

Dr. Arun Kumar Jain

1. Invited Talk entitled "Basics of EDX Analysis" during workshop cum Seminar on Microscopic Techniques in Nano Science organized by Department of Physics, Himachal Pradesh University, Shimla from March 30th to April 5th, 2011

2. Delivered Invited talk entitled "Application of TEM in Pathology" at Workshop on Techniques in Electron Microscopy Organized by Star College Project, Ramjas College; Department of Biotechnology and Electron Microscope Society of India at Ramjas College, University of Delhi from 20th to 22nd June 2011

3. Member, National Organizing Committee, International Conference on Electron Nanoscopy and XXXII Annual Meeting of EMSI held at Ramoji Film City Hyderabad from July 06 to 08, 2011.

4. Delivered invited talk entitled "How relevant is Transmission Electron Microscopy in Diagnostic Pathology" at International Conference on Electron Nanoscopy and XXXII Annual Meeting of EMSI held at Ramoji Film City Hyderabad from July 06 to 08, 2011

5. Chaired Scientific Session in Biological Science at International Conference on Electron Nanoscopy and XXXII Annual Meeting of EMSI held at Ramoji Film City Hyderabad from July 06 to 08, 2011

6. Attended Symantec Seminar on Endpoint Protection 12 held at Hotel Intercontinental Eros, New Delhi on 3rd August 2011

7. Appointed Member of the Facility Management Committee (FMC) for the Sophisticated Analytical Instrument Facility (SAIF), AIIMS, New Delhi

8. Delivered invited talk entitled "Diagnostic Application of TEM" at National Workshop cum Seminar on Advances in Electron Microscopy and Allied Fields - NWAEMA-2011 organized by Departments of Physics and Chemistry, Shoolini University, Bajhol, Solan from Sept. 23- 29, 2011

9. Attended 30th Meeting of the Facility Management Committee of the AIIMS-SAIF for Electron Microscopy held at AIIMS, New Delhi on 18th October 2011.

10. Participated in "Advance in Electron Microscopy in Virology research and 3D Imaging: The Road Ahead"held at National Institute of Virology, Pune on 20th and 21st October 2011

11. Attended ICMR Awards Presentation Ceremony and release of Commemorative Postage Stamp on ICMR held at Dr. D. S. Kothari Auditorium, DRDO Bhawan, Dalhousie Road, New Delhi on 8th Nov. 2011

12. Attended ICMR Centenary Symposium and Commemorative Currency Coin Release ceremony held at NASC Complex, ICAR, Pusa Road, New Delhi on 15th Nov. 2011

13. Attended 33rd Meeting of the Screening Committee for Registration of Public Funded Research Institutes / Universities held at DST, New Delhi on 17th Nov 2011

14. Delivered an Lead talk entitled "Understanding Disease Process: Electron Microscopy" during 1st Biennial National Conference of Indian Academy of Science and Nature held at Hotel Amar, Agra on 30th Nov. and 1st Dec. 2011.

15. Conducted World Health Organization sponsored training course for Advanced Level Technicians at National Institute of Pathology form Nov 24th to Dec. 23, 2011.

16. Attended International Symposium on Molecular Pathology organized by SRL Global Knowledge Forum, jointly sponsored by Association for Molecular Pathologists , USA; the Association of Indian Pathologists of North America and Molecular Pathology Association of India at Manekshaw Centre, New Delhi on 28th-29th January 2012

17. Participated in training program to post graduate students from Govt. Medical College, Amritsar from 6th to 7th Feb. 2012.

18. Attended International Speaker Program organized by M/s Pfizer on "Evolving Role of Biologics in the management of Ankylosing Spondylitis" by Prof. Pal Geher, held on 30.03.2012 at Hotel Taj Ambasssador, New Delhi

19. Member, Selection Committee, Department of Anatomy, VMMC and SJ Hospital, New Delhi for selection of Senior Research Fellow in ICMR-funded project

20. Reviewed STS project proposals for Short-Term Studentship program of ICMR (2011-12)

Dr. Usha Agrawal

1. Organizing Secretary of 26th Annual Conference Delhi Chapter, IAPM held on 17th April 2011 at VB Patel Chest Institute, North Campus, New Delhi.

2. Attended conference on 'IPCON-2011' organized by the Proteomic Society of India and Jawaharlal University from 4th-6th April 2011 at Hotel Parkland, New Delhi.

3. Attended 'Data Curation and Bioinformatics Workshop' organized by Shodhaka-ICMR-NIRRH at Mumbai from 31st May to 3rd June 2011.

4. Deputed to visit Tissue Repository Facility at ACTREC, Khargar, Navi Mumbai on 6th June 2011.

5. Facilitated the inspection for renewal of DNB Accreditation in National Institute of Pathology on 20th July 2011 by Dr Rajiv Sen, PGIMS, Rohtak.

6. Served as expert on Selection Committee for Research Associate (Pathology) held on 22nd July 2011.

Dr. LK Yerneni

1. Attended Workshop on Cytogenetics & Automated Slide Scanning Workstation conducted by Carl Zeiss, India on 5th May 2011, at Hotel Parkland, New Delhi.

2. Attended a Public Consultation meet on stem cell guidelines for northern region held on 17th December 2011 at Chinmaya Mission auditorium, New Delhi.

3. Attended several rounds of discussions at ICMR Headquarters during 2011 held amongst NRDC, ICMR and a Hyderabad based pharmaceutical company regarding transfer of epidermal culture technology to industry.

4. Visited a Pharma industry, Hyderabad, on 19th October 2011, as an inventor member of an ICMR-NRDC team to ascertain the suitability of their laboratories for undertaking the epidermal culture work towards clinical trial and the subsequent commercial exploitation of the technology.

Dr. AP Singh

1. Presented a paper titled *'Clinicopathological correlations in leprosy type 1 and type 2 reactions'* at 26th Annual IAPM- Delhi Chapter Conference held on 17th April 2011 at National Institute of Pathology-ICMR, New Delhi.
2. Participated in 6th International CME in Dermatopathology from 11th-13th November 2011 at India Habitat Center, New Delhi.
3. Delivered an invited talk on *'Tissue microarray in pathology'* at CME in Pathology held on 8th October 2011 at Maulana Azad Medical College, New Delhi.
4. Presented a paper titled *'Immunohistochemical expression of chemokine receptor CXCR3 and and its ligand CXCL10 in gliomas'* at Annual IAPM Delhi Chapter, IAPM Conference held at Maulana Azad Medical College on 25th February 2012.

Dr. Saurabh Varma

1. Attended Workshop on Cytogenetics workstation at Hotel Parkland, Safdarjung Enclave, New Delhi on 5th May 2011.
2. Participated as faculty for Workshop titled "Foundation workshop in clinical and laborartory medicine research" for UG medical students from 26th-28th July 2011 at NIP.
3. Participated as Faculty in WHO Training for Advanced Technical Course for the Technicians from 24th Nov. 2011 to 23rd Dec. 2011.
4. Attended and presented project before ethical committee on 21st December, 2011 Dean office, MAMC, Delhi
5. Lecture presentation and training for undergraduate students (4) of MBBS from Dept of Pathology, Govt Medical College Amristar on 8th Feb. 2012. The training was on flowcytometer.
6. Examiner of viva-voce of PhD students at NIP.
7. Lecture presentation and training session for the Post-Graduate (MD Pathology) students, Department of Pathology, Govt Medical College, Amritsar on special techniques. FlowCytometer and CLSM at NIP on 15th February, 2012.

Dr. AK Mishra

1. Faculty for the course no. BIO-G532 'Biostatistics and Biomodelling' under the off-distance Campus Ph.D. programme of National Institute of Pathology (ICMR) in collaboration with BITS, Pilani for the academic session 2011-2012.

2. Faculty as Co-supervisor for DNB (Pathology) thesis dissertation work: Role of epithelial mesenchymal transition in progression of bladder cancer: An immunohistochemical analysis. Course successfully completed by the candidate.

3. Involved as Co-Investigator in the extramural research project entitled, 'Immunogenetic profile of NPC– Nasopharygeal Cancer in a high prevalence region of northeast India', funded by Department of Biotechnology, Ministry of Science of Technology, Government of India.

4. Involved as Co-Investigator in the extramural research project entitled, 'Epigenetic studies in esophageal cancer in high risk region of north-east India' funded by Department of Biotechnology, Government of India, Ministry of Science of Technology.

5. Submitted application as a candidatureship for American Cancer Society for Beginning Investigator (ACSBI), to Union for International Cancer Control (UICC) for the research proposal entitled, 'Designing and implementing community based breast cancer screening programme for early detection and prevention and downstaging in northern part of India.

6. Served as a resource person for the 'National Seminar on Scientific Writing', organized by Bakson Homoeopathic Medical College and Hospital, Greater Noida, sponsored by Commission for Scientific and Technical Terminology, Ministry of Human Resource Development, Government of India on 2nd March 2012.

7. Attended one-day workshop on 'Application of Next Generating Sequencing (NSG) in Medical Research (ICMR)', held jointly by National Institute of Pathology (ICMR) and ICMR at NIP on 28th February 2012.

8. Attended lecture on 'Application of algebraic statistics in statistical genetics' by Professor MB Rao, University of Cincinnati, USA at All India Institute of Medical Sciences (AIIMS) on 9th December 2011.

9. Served as Faculty for the World Health Organization (WHO)-sponsored Training Course for Technicians and Advanced level held at National Institute of Pathology (ICMR) from 24th November 2011 to 23rd December 2011 for the topic, 'Basic Biostatistics and Epidemiology'.

10. Served as faculty for the Foundation Workshop on Clinical and Laboratory Medicine Research, held at National Institute of Pathology (ICMR) from 26th-28th July 2011 and delivered talk on '*Statistical Softwares'.*

11. Contributed to the research methodology towards proposal development for the research project entitled 'Cholinergic and non-cholinergic toxicity in tea-garden workers due to organophosphate pesticide exposure during pregnancy and its correlation with expression of serotonin receptors, SERT and PON1', submitted to ICMR for extramural grant.

12. Contributed to the research methodology towards proposal development for the research project entitled, 'Study of genetic alterations in extracellular matrix related genes in Chronic Obstructive Pulmonary Disease (COPD)', submitted to ICMR for extramural grant.

PDF SCHOLARS

Dr. Anurupa Chakraborty

1. Attended Workshop on Basics of Cell Culture and Stem Cell Techniques, DBT MSUB- ILSPARE Vadodara, 28th February - 3rd March 2012.

Ph.D. STUDENTS

1. Ms Vasundhra Bhandari participated and presented work in Lab steering committee meeting of EC funded Kaladrug project held at Strathclyde University, Glasgow, UK during 27 Jun-2 Jul, 2011.

2. Ms. Regina Devi Thoudam presented poster in American Association of Cancer Research (AACR) International Conference on 'New Horizons in Cancer Research: Biology to Prevention to Therapy', 13th-16th December 2011, Gurgaon.

3. Mr. Sandeep Verma presented poster titled, *'Role of Parasite Surface Antigen-2 gene of Leishmania donovani in antimony resistance'* in 'International Conference of Human Infectious Disease and Immunity' held during 14th-15th October 2011 at Karunya University, Coimbatore.

4. Ms Arpita Kulshreshtha presented poster on *'Transcriptome profiling for identification and characterization of miltefosine resistance associated genes in Leishmania donovani'* at MICROCON-2011 conference organized by Indian association of Medical Microbiologists held at BHU, Varanasi from 23rd-26th November 2011.

5. Mr. Avishek Srivastava attended 'Basic Course training on Flow Cytometry' conducted by BD Biosciences, from 4th-6th April 2011 at Gurgaon.

6. Ms Nitu Kumari attended the Indian Proteomics Conference and Post-Conference Hands-on Workshop at JNU, New Delhi during 4th-9th April 2011.

7. Ms. Nitu Kumari attended the Bio-Plex User Advance Training on 10th August 2011 at Bio-Rad House, Gurgaon.

8. Ms. Asheema presented poster titled, *'Association between polymorphism of xenobiotic-metabolizing genes and the risk of acute leukemia'* at AACR's first conference in India 'New Horizons in Cancer Research: Biology to Prevention to Therapy' held at Gurgaon during 13th-16th December 2011.

9. Ms Namita Singh attended IPCON-2011 'The Indian Proteomics Conference' at JNU, New Delhi during 3rd-5th April 2011.

10. Ms Namita Singh participated in "Hands-on Workshop on Proteomics Techniques" at JNU, New Delhi during 6th-8th April 2011.

11. Ms Priya Prasad attended IPCON-2011 'The Indian Proteomics Conference' at JNU, New Delhi during 3rd-5th April 2011.

12. Ms Priya Prasad presented poster titled, *'Role of superoxide dismutases in spontaneous aborters infected with Chlamydia trachomatis'* at International Symposium on Molecular Pathology held in New Delhi during 28th-29th January 2012.

13. Mr. Praveen Kumar attended Indian Proteomic conference on "Trends in Translational Proteomics" organized by Proteomics Society (India) in collaboration with JNU, New Delhi during 3rd-5th April 2011.

14. Mr. Praveen Kumar attended hands-on workshop on 'Basic Molecular Biology Techniques Relevant to Cancer Research' at ICPO (ICMR), Noida during 8th -12th August 2011.

15. Mr. Praveen Kumar attended lecture entitled "Rheumatoid arthritis- The search for next generation therapies" by Dr. Anwar Murtaza (Senior Scientist III, Worcester, USA) at AIIMS, New Delhi on 3rd November 2011.

16. Mr. Praveen Kumar attended CME on 'Diagnosis of autoimmune disorders: The continuing dilemma' at GB Pant Hospital, New Delhi on 10th-11th December 2011.

17. Rishi Man Chugh, attended workshop on cytogenetics & automated slide scanning workstation conducted by Carl Zeiss, India on 5th May, 2011, at Hotel Parkland, New Delhi.

18. Madhusudan Chaturvedi, attended workshop on cytogenetics & automated slide scanning workstation conducted by Carl Zeiss, India on 5th May, 2011, at Hotel Parkland, New Delhi.

19. Rishi Man Chugh, Madhusudan Chaturvedi & Hemlata Chauhan, attended workshop on intellectual property & innovation management in medical research on 11th-13th Nov., 2011, jointly organized by ICMR & NRDC at New Delhi, India.

20. Hemlata Chouhan, Rishi Man Chugh & Madhusudan Chaturvedi, attended a public consultation meet on stem cell guidelines for norther region held on 17th Dec. 2011 at Chinmaya Mission Auditorium, New Delhi.

DNB/ Ph.D. PROGRAMME

DNB PROGRAMME

The Post-graduate Level Training Programme in the speciality of Pathology continued during 2011-12. During the year, the following four students who appeared for the DNB Exam held in 2011-12 have come out with flying colours:

1. **Dr. Sherry Khan**
2. **Dr. Ila Jain**
3. **Dr. Disha Arora**
4. **Dr. Manpreet Bhatia**

As per guidelines of the National Board of Examination, the Institute conducted Review Examinations of the DNB students in July 2011. **Dr. Rajiv Sen**, PGIMS, Rohtak, appointed as Inspector by NBE came as DNB reviewer to NIP.

Ph.D. PROGRAMME

- **Mr. Gajender Katara** submitted his Ph.D. thesis on *"Studies on host immuno-determinants modulated during active disease in kala-azar and post kala-azar dermal leishmaniasis"* in 2011.
- **Mr. Pradeep** submitted his thesis on *"Genetic alterations and multidrug resistant gene expression profile of acute leukemia"* in February 2012.
- **Ms Regina** submitted her thesis on *"Genetic analysis and gene expression profile of gastric cancers in high risk region of north-east India"* in March 2012.
- **Mr. Dhirender** submitted his thesis *"Genetic variations and gene expression profiling of oral cancer associated with tobacco correlation"* in March 2012.
- **Ms. Rashmi Tomar** submitted her Ph.D. thesis on "Health Hazards of Phthalates vis-à-vis Idiopathic Male Infertility" in September 2011.

- **Mr. Ravi Deval** submitted his Ph.D. thesis on "Assessment of Cadmium and Lead toxicity on IUGR and its association with Growth Factors (VEGF and PlGF)" in 2011

During 2011-12, National Institute of Pathology further attracted young researchers- Research scholars with CSIR Junior Research Fellowships (1), and one woman scientist joined the Institute during this period.

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 of Raja Ramanna Fellow Chairperson, Scientific Advisory Committee, National Institute of Pathology, 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. Subhroto Sinha, Professor**, **Director,** National Brain Research Centre (NBRC), Near NSG Campus, Nain Mode, Manesar, Gurgaon, Haryana-122050	Member
6	**Dr. N.K. Mehra, Professor & Head,** Department of Transplant Immunology & Immunogenetics All India Institute of Medical Sciences, Ansari Nagar, New Delhi – 110 029.	Member
7	**Dr. R.R. Bhonde, Professor & Dean,** Manipal Institute of Regenerative Medicine (MIRM), 10, Service Road, Near Union Bank, Domlus Layout, Bangalore - 560071	Member
8	**Dr. Ravi Sirdeshmukh, Scientist** **Distinguished Scientist & Ass. Director,** Institute of Bioinformatics, Unit-1, Discoverer, 7th Floor, International Tech. Park Ltd. Whitefield Road, Bangalore-560 066	Member
9	**Dr. Sudha Bhatacharya, Professor & Dean,** Dept. Environmental Sciences, Jawahar Lal Nehru University, New Delhi.	Member

10	**Dr. Dhananjaya Saranath,** 601-B, Kalpak Gulistan, 9A, Perry Cross Road, Bandra West, Mumbai – 400 050	Member
11	**Dr. Kiran Katoch, Director** National Jalma Institute of Leprosy & Other Mycobacterial Diseases, Tajganj, Agra, UP	Member
12	**Dr. Chandrima Saha, Deputy Director,** National Institute of Immunology Aruna Asaf Ali Marg, New Delhi	Member
13	**Dr. Syamal Roy** Indian Institute of Chemical Biology, Council of Scientific & Industrial Research, 4, Raja SC Mullick Road, Kolkata – 700 032., West Bengal.	Member
14	**Dr. Ashok Sehgal** **Director, In-charge,** Institute of Cytology & Preventive Oncology, Research-cum-clinical complex, 1-7, Sector-39, Near Degree College, NOIDA, UP - 201301	Member
15	**Dr. Vijay Kumar, Scientist F & Head,** Division of BMS, Indian Council of Medical Research, New Delhi	Member
16	**Dr. Ashwini Kumar, Head** Indian Institute of Toxicology Research, Post Box No. 80, Mahatma Gandhi Marg, Lucknow – 226 001	Member
17	**The Principal** Vardhman Mahavir Medical College, Safdarjang Hospital, New Delhi – 110 029	Member
18	**Dr. B D Athani** Addl. DG & Medical Superintendent, Safdarjang Hospital, New Delhi	Member
19	**Dr. Sunita Saxena, Director** National Institute of Pathology, New Delhi	Member Secretary


INSTITUTIONAL
ANIMAL
ETHICAL
COMMITTEE

INSTITUTIONAL ANIMAL ETHICAL COMMITTEE

NIP Members

- Dr. Sangita Rastogi, Scientist 'E' & InCharge, Animal House, NIP (Member Secretary)
- Dr. Poonam Salotra, Scientist 'E', NIP (Member)
- Dr. Usha Agrawal, Scientist 'E', NIP (Member)
- Dr. Lakshmana Kumar Yerneni, Scientist 'D', NIP (Member)

External Members

Scientist Member

Dr. Harmeet Singh Rehan, Professor & Head, Department of Pharmacology, LHMC (Chairman)

Social Scientist Member

Dr. BB Batra, CGHS

Veterinary consultant

Dr. PK Yadav, Sr. Veterinary Officer, Experimental Animal Facility, AIIMS

CPCSEA Nominee

Dr. DN Rao, Professor, Department of Biochemistry, AIIMS (Main CPCSEA Nominee)
Dr. Smriti Rekha Dutta (Link CPCSEA Nominee)


INSTITUTIONAL
COMMITTEES

INSTITUTIONAL COMMITTEES

Purchase Committee

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

Transport Committee

Dr. Poonam Salotra
Dr. Purnima Paliwal
Dr. BS Raju
Ms. R. Saratha
Mr. Raja Ram

Library Committee

Dr. Sujala Kapur
Dr. Saurabh Varma
Dr. Usha Agrawal
Dr. L K Yerneni
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
Mr. Yogendra Kumar

Condemnation Committee

Dr. Ranvir Singh
Dr. Saurabh Varma
Dr. Avninder Singh
Dr. B S Raju
Mrs. R. Saratha
Mr. V.K. Khanduja

Tender Committee

Dr. Sangita Rastogi
Dr. Saurabh Varma
Dr. Ranvir Singh
Mrs. R Saratha
Mr. V.K. Khanduja

Renovation/Building Committee

Mr. B. Bose
Mr M.M. Prasad
Mr. AK Srivastava
Dr. Poonam Salotra
Dr. Sangita Rastogi
Dr. S Kapur
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

Technical Committee

Dr. Ashok Mukherjee
Dr. S N Das
Dr. Poonal Salotra
Dr. A.K. Jain
Dr. L.K. Yerneni
Mrs. R. Saratha
Mr. V.K. Khanduja

Canteen Committee

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


REFERRAL
SERVICES

REFERRAL SERVICES

HISTOPATHOLOGY AND CYTOLOGY LABORATORY

Histopathology and cytology diagnostic services were provided to patients from Safdarjung Hospital. Special diagnostic techniques using ancillary techniques such as histochemistry, Immunoflourescence, frozen section, immunohistochemistry for ER, PR, Her2neu and other diagnostic markers were routinely performed wherever relevant. The samples included 4671 cases over the past one year and included tumors from breast, oral cavity, urinary bladder, prostate and soft tissue. Other samples included renal biopsies, skin biopsies, bone marrow biopsies and lymphomas. New antibodies for assisting in diagnosis such as PGP1 and AMACR have also been procured and standardised.

Staff: Dr. Usha Burra, Dr. Avninder Pal Singh, Dr. Purnima Paliwal, Mrs. Karuna, Mrs. Krishna, Mrs Jyoti, Mrs. Shardha, Mr, Satpal, Mr, Anil, Mr. Shyamsunder, Mr. Rajsingh

MOLECULAR BIOLOGY LABORATORY

In the year under report, a total of 150 clinical samples were received from VL patients (blood, bone marrow), PKDL patients (blood, tissue biopsy and slit aspirates) and CL patients (tissue biopsy). Samples were processed for detection and species identification of *L. donovani* / *L. tropica* infections by PCR, PCR-RFLP, LAMP, Q-PCR and rk39 strip test.

Staff: Dr Poonam Salotra, Dr Ruchi Singh, Mr. Sandeep Verma, Mr. Gajendra Kumar Katara, Ms Arpita Kulshrestha, Ms Vasundhra Bhandari, Ms Vanila Sharma, Mr Himanshu Kaushal, Kumar Avishek, Mr Deepak Kumar Deep, Ms Kamlesh Sharma, Mr. Anish Saxena, Mr RC Chhetri.

CELL BIOLOGY LABORATORY

The Cell Biology lab. is primarily involved with translation of an innovative and cost-effective process of growth arrest of fibroblasts into graftable human epidermis *in*

vitro. So far, preliminary clinical experience in Burns patients using cultured epidermal autograft grown over growth-arrested NIH 3T3 feeders was highly promising and further clinical utilization of this technology is being planned as co-development project through partnership with a private sector Pharma industry. Currently, the lab. is also developing synonymous technical strategies to culture human epidermal autografts using other mouse as well as human fibroblasts as feeders. Additionally, the laboratory is aiming to understand the underlying molecular events in the developed techniques employing organotypic 3-D skin culture techniques.

The various technical capabilities at Cell Biology lab. are:

- Cell and Tissue culture, Colony forming assay and plating efficiency, Karyotyping, Histological, and Immunofluorescent Immunohistochemical methods
- 3-D dermal equivalent using scaffolds & Bioengineered models of skin
- Microbial culture methods and detection of *Mycoplasma* in cell culture
- *In vitro* quantification assays for cell proliferation by BrdU labeling and cells expressing specific markers using Image Analysis software
- Quantification of biomolecules by spectrophotometry
- Electron Microscopy - Scanning and Transmission
- PAGE & SDS-PAGE, PCR & agarose gel electrophoresis, RT-PCR & Western Blot

Staff : Dr. Lakshmana K.Yerneni, Mr. Bijender Kumar, Mrs. Charanjeet Kaur, Mr. RishiMan Chugh, Mr. Madhusudan Chaturvedi, Ms. Hemlata Chouhan, Mr. Dharmender Singh.

ELECTRON MICROSCOPE DIVISION

The EM Facility at National Institute of Pathology is a centralized core laboratory which provides High Resolution Analytical Transmission Electron Microscopy Application for biological tissues to all users. The facility is used for diagnostic as well as basic research activities by several scientists of the Institute.

The facility also provides EM research support to other researchers at regional and national level.

The Electron Microscopy Division is equipped with state of art 120kV Hitachi H7500 High Resolution Transmission Electron Microscope with side-mount Gatan and bottom-mount AMT digital camera systems along with Thermo Noran Energy Dispersive Microanalysis System. The processing laboratory is equipped with latest ultra-microtomes and cryo-ultra microtomes for cutting semi and ultrathin sections, Glass knife makers, Diamond Knives and can process tissues samples for both scanning as well as transmission electron microscopy.

Staff: Dr. AK Jain, Mr. Banajit Bastia, Mrs. Asha Rani Srivastava, Mrs. Anita Bhatia, Mr. Suresh

ENVIRONMENTAL TOXICOLOGY DIVISION

The Environmental Toxicology division at National Institute of Pathology is a centralized core laboratory with capabilities of High Resolution Mass Spectrometry in real time. The division is equipped with JEOL Direct Analysis in Real Time (DART) Time Of Flight mass spectrometer, Shimadzu GC 2014 Gas Chromatograph with Flame Ionization and Electron Capture Detectors along with Auto Sampler and Shimadzu High Performance Liquid Chromatography with UV and PDA detectors and Auto Sampler facilities. The division also has an ARL Backman Direct Current Plasma Spectrometer for trace metal analysis, Microwave Sample Digestion and Extraction Systems.

The division is undertaking extensive research in Human Environmental Biomonitoring Program and is involved in monitoring of toxic metals, phthalates and organic pollutants in human samples. The institute has developed a modest Environmental Specimen Bank with over 200 placental Specimens.

Staff: Dr. AK Jain, Dr. Nida Akhtar, Ms. Rashmi Tomar, Mr. Ravi Deval, Mr. Shashi N Kumar, Mr. Manoj

MICROBIOLOGY LABORATORY

The focus of studies in the Microbiology laboratory is on the molecular mechanism of immunopathogenesis of genital chlamydiasis. During the reporting period, research projects were continued in clinical collaboration with Department of Obstetrics & Gynecology, Safdarjung hospital, New Delhi. Endometrial curettage tissue was collected

from 75 spontaneous aborters undergoing D&E while blood samples were collected from 81 ANC patients (<20 wks.) for the diagnosis of *C. pneumoniae*. Another focus of research in the Microbiology laboratory is on *Chlamydia*-induced reactive arthritis, wherein, 33 arthritic patients were further enrolled for the collection of synovial fluid and blood for studies pertaining to intra-articular *C. trachomatis* infection. This work was carried out in clinical collaboration with Army hospital (R&R), New Delhi.

Staff: Dr. Aruna Singh, Dr. Sangita Rastogi, Mr. Praveen Kumar, Ms Namita Singh, Ms Priya Prasad, Mrs. Madhu Badhwar, Mrs. Asha Rani, Mr. Ajit Singh Lehra, Mr. Kamal Dev

ANIMAL HOUSE FACILITY

(CPCSEA Registration number: 102-1999/CPCSEA; Date of Registration: 28.04.1999)

The Animal House serves as a small animal Facility to the Institute's scientific community for conducting animal experimentation in accordance with the guidelines of **Committee for the Purpose of Control & Supervision of Experiments on Animals (CPCSEA).** The Institute has an **Institutional Animal Ethics Committee (IAEC)** as per the CPCSEA guidelines. During the reporting period, the scientists of the Institute obtained ethical clearance for their respective research projects from IAEC in its meeting dated 23rd August 2011.

Staff: Dr. Sangita Rastogi, Mr. Kuldeep Kumar Sharma, Mr. Madan Lal, Mr. Bala Dutt.

FLOWCYTOMETRY LABORATORY

In total, **1275** samples of blood, cervical cells, leukaemic patients, urinary bladder samples were acquired and analysed for surface antigen expression, intracellular cytokine response and apoptosis.

Scientific Staff: Dr Aruna Mittal. Dr. S. Verma, Mr. P.D. Sharma

CONFOCAL LASER SCANNING MICROSCOPY LABORATORY

Total of **147** images were acquired on confocal laser scanning microscope.

Scientific Staff: Dr S. Saxena, Dr. S. Verma, Mr. P. D. Sharma

LIBRARY

The Institute library continues to cater to the needs of the Institute's staff, students and the research staff by subscribing to **18** (print) and **15** (Indian) journals. **55** Online Journals are subscribed through M/s Wiley's and Scince Direct. The faculty of Safdarjung hospital and other Institute's Library are also using the facility. The library serves the scientific staff, DNB, MSc, Ph.D. students, WHO fellows and trainees by getting books and other Library services. The users access the online journals and reprints through inter-library loan from Safdarjung hospital library, NIC, National Medical Library, INSDOC and other ICMR Institutes as requested by the scientific and administrative staff of the Institute. The library also receives inter-library loan request on email to send photocopies of the same by post free of cost. The photocopy facility for the scientists and students is being continued.

The Institute library has more than **10,000** (Books, Bound Journals, CDs, Online Books, Thesis, Annual Reports, WHO and AFIP Fascicles) pertaining to Pathology, Cancer, Computer Science, Immunology, Infectious Diseases, Toxicology, Statistics, Electron Microscopy, Confocal Microscopy, sub-branches of Pathology and other specialized medical subjects and Hindi books have also been added to the Library collection, Central News Agency (In-print and Online through Sc.-Direct) and PUB-MED, NML-ERMED, JCCC@ ICMR (consortium between NML ICMR and AIIMS) and other online services are also provided for the scientific and technical staff of the Institute. JCCC and J-Gate Custom content for Consortia through informatics India Ltd. is used for resource sharing of other ICMR Libraries. The facility allows the user to be more flexible in meeting their needs. The library also provides other services like indexing and abstracting of books and journals. The library displays the recent scientific publications, Annual Reports, Newsletters of other Institutes and newspaper clippings of scientific, technical and govt. policies.

Local Area Network (LAN) facility with six nodes terminal internet connection has been installed in the library for internet browsing and e-mail access. Online journals which are subscribed from Science-Direct, Wiley, ICMR Consortia, NML-Ermed can be accessed by scientists on their desktop (6 terminals).

The IOP Newsletter (Quarterly) in both English and Hindi is being continued since 2003 and is being published and distributed.

E-Mail: ioplibrary@rediffmail.com

Staff: Dr. S. Kapur, Mrs. Anita Sharma, Mrs. Sangeeta Batra, Mr. Dharmender

COMPUTER DIVISION

Computer Division of the Institute serves as the backbone for communication by all other divisions and department of the Institute through Local Area Networking and proving internet services. The Computer Division is equipped with three severs for LAN, antivirus and application programs along with latest computer systems, a laser printer, color inkjet printers, scanner and up-to-date software. The department helps the students in conducting weekly journal club meetings as well as in data entry and formatting of thesis. Through the maintenance of Histopathology software, it helps in recording, storage and archival of histopathology data.

The division helps in the compilation and generation and printing of Annual Report, Highlights and other documents. The computer division also takes care of day to day assistance and maintenance of almost 70 computers in different divisions of the Institute. Computer Division is also responsible for maintaining the Institute's website, uploading latest information and updating the different web pages.

It is actively involved in financial accounting, i.e generation of pay, paybills, schedules, pay slips, compilation of income tax, filing of quarterly and annual tax returns, etc.

Staff: Dr. AK Jain, Mr. Shiv Prakash, Ms. Seema Sharma


STAFF
LIST

STAFF LIST

SCIENTIFIC STAFF LIST

1.	Dr. Sunita Saxena, MBBS,DCP, MD (Path)	Director
2.	Dr. Sujala Kapur, MBBS, MD (Path & Microbiology)	Scientist 'E'
3.	Dr. Sangita Rastogi, M.Phil., Ph.D.	Scientist 'E'
4.	Dr. Poonam Salotra, M.Sc.(Biochem), Ph.D. (Biochem)	Scientist 'E'
5.	Dr. AK Jain, M.Sc.(Dairy Bacteriology), Ph.D.	Scientist 'E'
6.	Dr. Usha Agrawal, MBBS, MD (Path) (w.e.f. 25/03/2011)	
7.	Dr. Nasreen Z. Ehtesham, M.Sc., Ph.D.	Scientist 'E'
8.	Dr. Ranvir Singh, MBBS (Upto 03/01/12)	Scientist 'D'
9.	Dr. AK Bagga, MBBS (Upto 01/09/11)	Scientist 'D'
10.	Dr. LK Yerneni, M.Sc., Ph.D.	Scientist 'D'
11.	Dr. Anju Bansal, MBBS, MD (Path)	Scientist 'C'
12.	Dr. Saurabh Verma, M.Sc., Ph.D.	Scientist 'C'
13.	Dr. Purnima Paliwal, MBBS, MD (Path)	Scientist 'C'
14.	Dr. Avninder Pal Singh, MBBS, MD (Path)	Scientist 'C'
15.	Dr. Ruchi Singh, Ph.D. (w.e.f. 19/01/2012)	Scientist 'C'
16.	Dr. S. Appala Raju Bagadi, M.Sc.(Statistics), Ph.D.	Scientist 'B'
17.	Dr. Poonam Gautam, Ph.D. (w.e.f. 01/02/2012)	Scientist 'B'
18.	Dr. AK Misra, M.Sc. (Statistics), (Ph.D. (Statistics) (upto 08/06/2012)	Scientist 'B'
19.	Dr. Aruna Singh, M.Sc., Ph.D.	Consultant

TECHNICAL STAFF

1. Mrs. Asha Rani Srivastava, Technical Officer "A"
   (superanutaed on 30/09/2011)
2. Dr. L.C. Singh, Technical Officer "A"
3. Mrs. Madhu Badhwar, Technical Officer "A"
4. Mr. Shiv Prakash, Technical Officer "A"
5. Mrs. Seema Sharma Technical Officer "A"
6. Mrs. Asha Rani, Technical Officer "A"
   (superanutaed on 28/02/2012)
7. Mr. Chandi Prasad, Technical Assistant
8. Mrs. Anita Bhatia, Technical Assistant
9. Mrs. Karuna, Technical Assistant
10. Mrs. Krishna, Technical Assistant
11. Mr. Kuldeep Kumar Sharma, Technician Assistant
    (w.e.f. 07/06/2011)
12. Mrs. Valsamma Mathew, Technician 'C'
13. Mr. Jagdish Pant, Technician 'C'
14. Mr. Pushp Raj, Technician 'C'
15. Mr. P.D. Sharma, Technician 'C'
16. Mr. Suresh Bhimrao Kamble, Technician 'C'
17. Mr. Satyapal Singh, Technician 'C'
18. Mr. Madan Lal, Technician 'C'
19. Mr. Shiv Bahadur, Technician 'C'
20. Mr. Dharampal, Driver
21. Mr. Sushil Kumar, Driver
22. Mr. Manwar Singh, Driver
23. Mr. Puran Singh, Driver

24. Mr. Rajender Kumar, Technician 'B'
(superanutaed on 30/11/2011)
25. Mr. Anil Kumar Verma, Technician 'A'
26. Mr. Bijendra Kumar, Technician 'B'
27. Sh.Kamal Dev, Technician 'B'
28. Mr. Shyam Sunder, Attendant (Services)
29. Mr. Ajit Singh Lehra, Attendant (Services)
30. Mr. Daya Sagar, Lab. Attendant (Services)

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. Sangeeta Batra, Jr. Librarian
16. Mrs. Sushma Ralhan, U.D.C.
17. Mrs. Sharmila Kamra, U.D.C.

18. Mrs. E. Sharda, Stenographer
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. Ms. Jyoti, LDC
24. Mr. Brijender Singh, LDC
25. Mrs. Archana, LDC
26. Mr. Bala Dutt, Attendant (Services)
27. Mr. Jagdish Ram, Attendant (Services)
28. Mr. Dharmendar Singh, Attendant (Services)
29. Mr. Ram Chander Das, Attendant (Services)
30. Mr. Puran Chand, Attendant (Services)
31. Mr. Rajendra Kumar, Attendant (Services)
32. Mr. Manoj Kumar, Attendant (Services)
33. Mr. Anish Kumar Saxena, Attendant (Services)
34. Mr. Raj Singh, Attendant (Services)
35. Mr. Sajid Hussain, Attendant (Services)
36. Mr. Chandrika Prasad, Attendant (Services)
37. Mr. Sanjay Dutt, Attendant (Services)
38. Mrs. Charanjeet Kaur, Attendant (Services)