



HIGHLIGHTS 2009-2010

INSTITUTE OF PATHOLOGY

(INDIAN COUNCIL OF MEDICAL RESEARCH) Post Box No. 4909, Safdarjung Hospital Campus, New Delhi - 110029





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THRUST AREAS OF RESEARCH

TUMOR BIOLOGY

In Tumor Biology Division, scientists are working on understanding molecular pathology, identification of diagnostic, predictive and prognostic biomarkers and drug targets for *breast, urogenital, hematological and brain cancers.* Investigation on the **gene-environmental link** responsible for very high incidence of several malignancies, especially those associated with tobacco and pesticide (oral, esophageal, gastric, lung and breast cancers) in north-eastern states in India, is also a major area for research. The field of cancer epidemiology has now emerged as one of the core disciplines.

1. Study on gene expression and hypermethylation profiles in early-onset breast cancer

Analysis of gene expression and hypermethylation profiles associated with early onset breast cancer is being done to elucidate the genetic factors associated with breast cancer in young women with identification of associated molecular mechanisms. Gene expression profiling in 18 cases identified about 300 differentially expressed genes in tumor tissue in early onset cases compared with the late onset cases. Human whole genome methylation analysis was performed on Illumina Human methylation27 chip with total of 24 samples using DNA from adjacent normal tissues as controls. Differential methylation analysis performed between early and late cases, showed total 5192 differentially CpG sites where 2395 were hypermethylated sites.

Preliminary data analysis has been carried out using the differentially expressed gene list, using DAVID Bioinformatics Resources 2008 package.



Classification of upregulated genes into various pathways

2. Establishment and characterization of cell lines from primary breast cancers

Cell lines provide an unlimited, self-replicating source of cells for studying cancer cell biology and for the development of new treatment strategies against cancer. During the year under report, amongst 36 breast tumours used for initiation of primary cultures, 23 primary cultures were established. Two breast cancer cell lines have been developed from primary breast tumours from young Indian women that may serve as a good *in vitro* model for understanding early breast tumorigenesis and mechanisms underlying breast tumorigenesis in Indian population. Both the cell lines have been characterized for expression of epithelial (CK, EMA), mesenchymal (vimentin) and biological (ER, PR, Cerb B2,p53) markers, cytogenetic characteristics, ultrastructural characteristics and capability for *in vitro* tumorigencity.



Purified culture of PCB20 (200X)



Ultrastructural analysis



In vitro tumorigenicity in soft agar

3. Type 1 growth factor receptor family : Expression and correlation with response to neo-adjuvant chemotherapy in locally advanced breast cancer

This study is undertaken to analyze the role of type 1 growth factor receptor genes EGFR, c-erbB-2, c-erbB-3 along with MDR1 and AR genes in predicting response to neo-adjuvant chemotherapy in locally advanced breast cancer cases.

Fifty five matched samples of pre- and post- NACT tumor tissues from locally advanced breast cancer patients including 39 responders and 16 non-responders to neo-adjuvant chemotherapy have been studied during the year under report. Total RNA (TRIzol method) was isolated and cDNA was generated using High Capacity cDNA Archive kit (*Applied Biosystems*) and relative quantitation of expression levels of EGFR, c-erbB-2, c-erbB-3, MDR1 and AR genes were assessed in pre- and post- NACT breast tissue by real time RT-PCR (ABI 7000 SDS, *Applied Biosystems*) using TaqMan probe assay. Significant high expression of AR gene (p 0.031) was found in pre- therapy tumor tissue in responders only suggesting its independent prognostic value.

4. Micro- satellite instability in androgen receptor gene and P53 polymorphism in prostate carcinoma in Indian males

Several genes involved in cell cycle regulation and apoptosis have been reported in relation to both prostate cancer development and disease progression as the altered activity of these genes may influence the risk of prostate cancer (CaP). This study investigates the role of

androgen signaling (AR, PSA) and metabolizing (CYP19) genes, p53 codon 72 polymorphisms and mutations/expression of mismatch repair genes MLH1 and MSH2 in genetic susceptibility and progression of prostate carcinoma. Significant association of short AR CAG repeats (<24) (OR 1.75(1.00-3.09), 0.05) and p53 codon 72 Pro/Pro genotype was found with CaP risk (Fig. 1). The results further showed that the GG genotype of PSA polymorphism at position–158 is playing a protective role against the risk of prostate cancer. A C/T transition at -93 position of the core promoter region of MLH1 gene was also identified (Fig. 2) and CC genotype was found as a genetic predisposing factor for prostate cancer development.



Fig. 1: Genotypic distribution at codon 72 polymorphism in p53 gene



Fig. 2: Electrogram showing the sequence variants -93 position of the core promoter region of MLH1 gene

5. Characterization of host immune profile associated with progression of superficial TCC of bladder by microarray analysis

According to the Delhi Cancer Registry, in 2003, bladder cancer was the 6th most common cancer, surpassed in frequency only by cancers of the lung, larynx, tongue, prostate, and esophagus. The majority of the patients present with superficial disease and are treated by transurethral resection of the bladder tumor. More than half of these patients experience

recurrence, with about 20% progressing to muscle invasive disease. The natural history of bladder cancer is largely unpredictable owing to tumor heterogeneity.

Earlier reports have shown that patients having bladder malignancies were immunodeficient manifesting as impaired cell-mediated and humoral immunity as well as by impaired non-specific host defence mechanisms. Identification of defects in host anti-tumour immunity responsible for recurrences can help predict tumor behavior. It would also help in understanding the biologic profile responsible both for recurrence and resistance to treatment.

This project was undertaken with the aim to identify host immune determinants for recurrence and progression of Superficial Transitional Cell Cancer (TCC) of bladder.

In the year under report, blood and urine samples from 38 cases of bladder cancer have been collected and tumour tissue was collected from 35 cases which include: 7 invasive and 28 non-muscle invasive tumours. RNA has been isolated from all the cases collected and cDNA conversion carried out as already mentioned. Quantitation was carried out using Nanodrop (*Thermo Scientific*) and RNA quality checked on agarose gel electrophoresis.

RT-PCR was performed on tumour and normal RNA for human Th1-Th2-Th3 cytokine/chemokine genes and the procedure was standardized and the results of the foldchanges are as depicted below. The results show that most of the chemokines (CCR2, CCR3, CCR5) and cytokines IL2, IL4, IL6, IL6R, IL9, IL13 and IL17A are down regulated while IL18, IL23A, IL13RA1, IL7 and IL1R2 are upregulated. Genes of the JAK-STAT pathway and MAP kinases are also upregulated.





6. To study the role of cyclooxygenases in cytokines dysfunction of invasive and non- invasive TCC of human bladder

Cyclooxygenase (COX) is responsible for inflammation, angiogenesis and tumour progression. Cyclooxygenases exist in two isoforms, viz. : Cox-1 and Cox-2. The latter are rate limiting enzymes in the formation of prostaglandins from arachidonic acid, are upregulated in multiple types of solid tumors, including urinary bladder in humans. Prostaglandin has been a major cox product involved in tumor development and progression. In this study, we evaluated the role of cyclooxygenases and associated cytokines in PBMCs and its gene expression in invasive and non- invasive TCC of human bladder.

Heparinized blood samples from normal healthy individuals and TCC patients from Urology Department at Safdarjung hospital were collected for the study of Cox-1 and Cox-2 gene expression using RT-PCR and their expression in correlation with cytokines studied on flow cytometer and CLSM. Out of total 33 cases, 3 cases were invasive while 30 were non-invasive. Out of 3 invasive cases, 1 (33.3%) and 2 (66.6%) were from grade II and grade III respectively while out of 30 non-invasive cases, 4 (13.3%), 19 (63.3%) and 7 (23.3%) were from grades I, II and III respectively.

Total RNA isolation was done from human biopsy tissues of TCC. The relative quantitation of expression levels of Cox 1 and Cox 2 genes was carried out by real time RT-PCR. Expression levels were normalized to individual GAPDH (internal control gene). Primers and probe for the target genes and internal control gene were taken.

Cox-2, Cox- 1 and GAPDH forward primer, reverse primers with their probes were used in experiments.

Out of 33 samples, 6 non- invasive cases that did not show consistency with internal control gene GAPDH were excluded. Cox-2 gene expression was found upregulated in all the 3 (100%) invasive cases and in 20 (83.3%) out of 24 non- invasive cases because 4 cases showed normal expression (Fig.1 - 2 are showing Cox-2 and Cox-1 gene expression).

The flow cytometric studies on separated PBMCs from TCC patients showed the increased expression of IL-1 β and IL-6 in comparison with normal healthy individuals. The mean percentage of double positive cells of IL-1 β and IL-6 along with Cox-2 increased from 18.3 ± 8.7 and 23.08± 7.32 to 41.7 ± 11.9 and 37.87± 6.14 respectively in cancer patients in

comparison to normal healthy groups. Cox 2 expression was seen in 3 (100%) invasive and in 18 (60%) out of 30 non-invasive TCC samples using immunofluoresecence. Cox 1 expression was frequently seen in both type of samples.



Fig.1: Showing Cox-2 gene expression



Fig. 2: Showing Cox-1 gene expression.

7. Role of tobacco and pesticides use in causation of cancer in north-east India

A very high incidence of cancers particularly those associated with the use of tobacco and pesticide exposures has been reported in north-east region in India. The type and pattern of tobacco and betel nut use in this region is different from the rest of the country. There is an extensive use of pesticides in tea-gardens in north-east which can lead to widespread occupational and environmental exposures. Exposure alone is not sufficient to explain the high incidence of cancer in this region. Multicentric studies have been initiated in this region to find out if genetic factors, in addition to common environmental exposure and dietary habits could possibly explain the high prevalence. This study investigated patients from three collaborating PBCRs at Dr. B.B.C.I., Guwahati, Sir T.N.M. hospital, Gangtok and Civil hospital, Aizawl for polymorphisms in genes associated with xenobiotic metabolizing enzymes GSTT1 and GSTM1, CYP 19 and gene expression profile I patients of oral, esophageal, gastric and lung cancers and their association with tobacco consumption. This study establishes baseline frequency data for GST polymorphisms in this population, however, results do not support the hypothesis that GSTM1 and GSTT1 null genotypes increased risk of cancer in this population. No significant association of GSTM1 and GSTT1 null genotypes was found for oral and gastric cancer while they appeared to work as a protective factor for lung cancer.

Genome-wide analysis of chromosomal changes using the Affymetrix GeneChip® Human Mapping 10K Array Xba 142 2.0 single nucleotide polymorphism array was done for Copy Number Alterations (CNAs) in 20 pairs of matched germ line and frozen tumor DNA samples in esophageal cancer. CNAs were identified in several chromosomal regions. The most common sites for gain in ESCC were 3q and 5p. Regions on 3p, 8p, 13q and 18q were the most common sites for loss in ESCC. Some of these regions contain cancer genes known to be involved in ESCC and others hold genes that have a known role in other cancers but which have yet to be established as esophageal cancer genes. The amplified regions include gene pathways involved in cellular migration and proliferation, tumor cell metastasis and invasion, anchorage independent growth, inhibition of apoptosis and MAPK pathway. Deleted regions included genes involved in suppression of tumor including those involved in Wnt signaling and focal/ cell adhesion pathway.



Frequency plot of gains and loss throughout the genome in twenty patients with ESCC and exposed to tobacco and betel quid. Y-axis, the percentage of patients having gain (red, above 0) or losses (green, below 0) and X-axis, position on the chromosome

Investigation on the genetic changes associated with pesticide exposure, gene environmental interactions and their contribution in the occurrence of cancer showed no significant association of p53 codon 72 polymorphism, 5' UTR T>C polymorphisms in CYP 17 gene and GST M1, T1 null genotypes with breast cancer risk in this region, however, a positive association was observed with the GG genotype of GSTP1 gene. Mutations were detected in exon 27 of the BRCA2 gene in 3 cases.



8. Expression of fusion oncoprotein and gene expression profiling in acute and chronic leukemia

Diagnosis of Chronic Myeloid Leukemia (CML) is based on the detection of the classic cytogenetic abnormality, BCR-ABL gene or Philadelphia chromosome (Ph chromosome). This study had been undertaken to identify the genes associated with the presence of fusion genes using oligonucleotide microarray. Preliminary results of gene expression profile showed upregulation of MAPKKK cascade, G protein coupled receptor internalization, interleukin-1 beta secretion, regulation of T-cell differentiation, signal transduction by p53 class mediator resulting in induction of apoptosis, regulation of GTPase activity, cell proliferation and of I-kappaB kinase/NF-kappaB cascade. Downregulated genes included regulation of progression through cell cycle.

9. Expression of activator and target genes of Nuclear Factor-Kappa B (NF-kB) transcription factor in acute leukemia

Abnormalities in the regulation of NF- kB pathway are frequently seen in leukemias, however, activation is not uniform among AL patients. In our study, NF-kB pathway was considered "activated" when the gene expression level of IK-B gene was higher than the normal bone marrow sample. In ALL as well as in AML, CD34+ blasts showed higher expression of Bcl2 and lower expression of IkB and cIAP-2. In AML, but not in ALL, expression level of IkB- α was found to be lower in CD34+ blasts. Non- responder patients of AML showed lower p53 and higher IKK-alpha expression suggesting that loss of p53 function leads to transcriptional induction of IKK- α which results in NF-kB mediated gene expression. This appears to be an important mechanism for activation of NF- kB in AL. The study provides important insights to explain the pathogenetic mechanism involved in AL. The study has been concluded.

10. Identification of diagnostic and prognostic biomarkers in brain tumors: A tissue micro- array based approach

A high-throughput Tissue Microarray (TMA) chip containing 300 brain tumors (200 gliomas and 100 meningioma-schwwannoma)from archival paraffin blocks at IOP according to

histological grades based on WHO classification has been constructed using tissue arrayer with core diameter of 1.0 mm. Protein expression of some of the differentially expressed genes identified by DNA microarray at National Cancer Institute using TMA-IHC were analyzed for identifying potential diagnostic and prognostic biomarkers.



Fig. : Schwannoma-meningioma TMA stained with anti-nestin antibody.

PATHOLOGY OF INFECTIOUS DISEASES

CHLAMYDIASIS

1. Role of chlamydial heat shock proteins in pathogenesis of genital tract infection in women

There are number of studies that have been devoted to cell-mediated and humoral immune responses to cHSP60 and cHSP10, however, there is no study on their potential role in apoptosis of primary cervical epithelial cells that are privilege target for chlamydial infection. Thus, we investigated the potential role of chlamydial heat shock proteins (cHSP) 60 and cHSP10 in apoptosis of primary cervical epithelial cells.

To study the changes in mRNA expression of genes related to apoptosis in these epithelial cells, we employed a cDNA microarray approach human apoptosis expression array 205 different human genes related to apoptosis. In cHSP60 stimulated cells, 10 genes were significantly (p < 0.05) upregulated and 6 genes were significantly (p < 0.05) downregulated. Similarly, in cHSP10 stimulated cells, 11 genes were significantly (p < 0.05) upregulated and 4 genes were significantly (p < 0.05) downregulated (Table 1). To confirm the data, few genes of interest were subsequently quantified by real-time RT-PCR. We observed that interleukin (IL)-1 β -convertase, caspase-3, -8 and -9 which were upregulated in cDNA microarray were also upregulated in real-time RT-PCR upon stimulation with cHSP60 and cHSP10 suggesting thereby role in apoptosis.

Table 1: Modulation of gene expression in epithelial cells after stimulation with cHSP60 and	ıd
cHSP10 for 4 h.	

S. No.	Gene Description	Accession No.	cHSP60	cHSP10
1	Cyclin dependent kinsae 4	M14505	(-)8.3 <u>+</u> 0.32	(-)5.2 <u>+</u> 0.46
2	Cyclin-B1	M25753	(-)17.1 <u>+</u> 0.19	(-)4.6 <u>+</u> 0.24
3	Phospholipase D1	U38545	3.8 <u>+</u> 0.62	9.2 <u>+</u> 0.57
4	ERK-1	X60188	4.6 <u>+</u> 0.44	5.4 <u>+</u> 0.19
5	MAPK Kinase kinase 3	U78876	9.3 <u>+</u> 1.2	3.2 <u>+</u> 0.41
6	PCNA	M15796	(-)2.4 <u>+</u> 0.22	Unchanged
7	E2F transcription factor	M96577	(-)3.9 <u>+</u> 0.61	Unchanged
8	P53 induced protein	AF010315	4.1 <u>+</u> 0.2	Unchanged
9	Bcl-2 antagonist of cell death	U66879	Unchanged	3.1 <u>+</u> 0.12
10	IL-1 beta convertase	U13699	11.8 <u>+</u> 1.1	8.3 <u>+</u> 1.3
11	Caspase-3	U13737	4.5 <u>+</u> 0.64	5.9 <u>+</u> 0.22
12	Caspase-8	U60520	3.3 <u>+</u> 0.23	6.4 <u>+</u> 0.67
13	Caspase-9	U56390	7.1 <u>+</u> 0.19	4.6 <u>+</u> 0.32
14	TNF-receptor associated factor-6	U78798	3.1 <u>+</u> 0.91	5.7 <u>+</u> 0.81
15	FAAD-like apoptosis regulator	AF010127	2.2 <u>+</u> 0.33	9.7 <u>+</u> 0.41
16	TNF superfamily member 6	Z70519	Unchanged	1.8 <u>+</u> 0.11
17	TNF receptor family member 1B	M32315	(-)14.3 <u>+</u> 1.3	(-)7.2 <u>+</u> 0.82
18	TNF receptor family member 10B	AF016268	(-)2.8 <u>+</u> 0.41	(-)6.6 <u>+</u> 0.36

The expression of genes was analyzed in epithelial cells of 3 women using an apoptosis cDNA expression array from *Clontech* containing 205 immobilized human cDNAs. Values in columns entitled cHSP60 and cHSP10 are representing normalized mean fold change \pm standard deviation after stimulation as compared to unstimulated control, (-) indicates fold downregulation and rest are fold upregulated genes.

2. Effect of sex hormones on induction of immunity by dendritic cells in female reproductive tract during Chlamydia trachomatis infection

Understanding the mechanism of DC antigen presentation and the role of sex hormones in modulating the immune responses during chlamydial infection will help in understanding of immunopathogenesis of *Chlamydia trachomatis*.

The enriched $CD14^+$ cells were cultured in the presence of 50 ng/ml Granulocyte Macrophage Colony Stimulating Factor (GM-CSF) and 20 ng/ml IL-4 for 6-7 days and were then washed and infected with live chlamydial EBs at an MOI of 2: 1 (DC: EB) for 6 hrs. In

parallel experiments, DCs were pretreated with estradiol at concentrations of 10µg/mL, lug/mL and 0.1µg/ml for 24 hrs and were then pulsed with chlamydial EBs. After exposure, total RNA was extracted from the cells and TLR signalling pathway genes were studied using Real Time PCR based arrays. Estradiol treatment significantly reduced the release of proinflammatory cytokines IL-12 and TNF- α at the highest concentration, however, a nonsignificant decrease in levels of IL-6 was observed. A significant increase in secretion of IL-10 was observed with highest E2 concentration (Fig. 1). The expression of a number of Toll-like receptor signalling genes such as IRAK4, MyD88 and nF-κB were upregulated following chlamydial infection. Gene expression of significantly expressed genes in estradiol treated and untreated conditions are shown in Fig. 2. Estradiol pretreatment significantly reduced expression of TLR4 as compared to untreated cells. Gene expression of TLR4 associated downstream signalling molecules as IRAK4 and nF-kB was also found to be downregulated. Gene expression of Th1 associated cytokines IL-12, IL-6, TNF- α and IFN- γ was also reduced, however, expression of IL-10 by dendritic cells increased significantly after estradiol pretreatment.



Fig. 1: Cytokine levels in supernatant of dendritic cells pulsed with Chlamydia. Significant reduction in secretion of TNF- α and IL-12 was observed. IL-10 levels increased upon increasing estradiol concentrations. No E2: estradiol untreated dendritic cells

3. Modulatory role of antichlamydial agents in Chlamydia trachomatis infection and their therapeutic potential

Chlamydia trachomatis infection induces a wide array of inflammatory cytokines, which may contribute to *Chlamydia*-induced pathologies. To date, studies of reproductive sequelae following azithromycin and doxycycline treatment for *C. trachomatis* infection and their effect on immune molecules like cytokines in humans are lacking. Hence in this study, we have evaluated the effect of azithromycin and doxycycline *in vitro* on cytokines in cervical cells stimulated with chlamydial Elementary Bodies (EB's).

In primary infection, a significant decrease in IL-1 β , IL-6, IL-8 and TNF- α was observed in Group I and Group II in presence of azithromycin in *C. trachomatis* stimulated cells (Table 1). In presence of doxycycline, a significant decrease in the levels of IL-1 β and IL-6 was observed in Group I whereas no significant decrease in the levels of cytokines was observed in Group II (Table 1).

In recurrent infection, a significant decrease in the IL-1 β , IL-6, IL-8, IL-10 and TNF- α was observed in Group I and Group II in presence of azithromycin in *C. trachomatis* stimulated cells (Table 2). In presence of doxycycline, no significant decrease in the levels of cytokines was observed in both the groups (Table 2).

		Gr	oup I (n=52)				Gro	up II (n=44)	(
	СT	CT + Azithromycin	P value ^a	CT + Doxycycline	P value ^b	CT	CT + Azithromycin	P value ^c	CT + Doxycycline	P value ^d
IL-1ß (pg/ml)	143 (22-490)	76 (15-396)	0.002*	73 (32-365)	0.01*	240 (52-657)	120 (25-381)	0.006*	175 (25-606)	0.1
IL-2 (pg/ml)	2 (UDL-38)	5 (UDL-26)	0.6	6 (UDL-37)	0.4	6 (UDL-42)	5 (UDL-36)	0.9	5 (UDL-20)	0.8
IL-6 (pg/ml)	77 (42-688)	48 (7-354)	0.002*	53 (20-428)	0.007*	217 (20-537)	114 (15-374)	0.001*	172 (22-383)	0.08
IL-8 (pg/ml)	193 (78-717)	63 (8-258)	<0.0001*	180 (30-584)	0.1	255 (110-966)	117 (30-448)	<0.0001*	244 (57-627)	0.1
IL-10 (pg/ml)	138 (24-692)	101 (23-512)	0.2	136 (28-448)	0.5	246 (48-819)	197 (37-739)	0.09	219 (56-654)	0.5
IL-13 (pg/ml)	5 (UDL-28)	6 (UDL-20)	0.9	6 (UDL-33)	0.9	7 (UDL-24)	5 (UDL-18)	0.2	6 (UDL-40)	0.9
IFN-y (pg/ml)	144 (21-611)	91 (14-488)	0.07	115 (11-437)	0.2	88 (20-574)	61 (22-524)	0.3	73 (15-446)	0.4
ΓNF-α (pg/ml)	185 (27-582)	94 (30-439)	0.01*	127 (12-522)	0.08	115 (18-389)	76 (15-277)	0.01*	87 (21-392)	0.2

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Cytokine concentration is denoted by median and range in parenthesis; UDL-Under detection limit

^a denotes significance level between CT+Azithromycin and CT in Group I;^b denotes significance level between CT+Doxycycline and CT in Group I;^c denotes significance level between CT+Azithromycin and CT in Group II; ^d denotes significance level between CT+Doxycycline and CT in Group II; ^{*} Denotes significance level

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	CT	CT + azithromycin	P value ^a	CT + Doxycycline	P value ^b	CT	CT + azithromycin	P value °	CT + Doxycycline	P value ^d
IL-18 (pg/ml)	177 (15-544)	74 (20-385)	0.03*	162 (28-414)	0.7	389 (36-1240)	189 (14-422)	0.02*	259 (28-440)	0.1
IL-2 (pg/ml)	4 (UDL-16)	6 (UDL-30)	0.5	6 (UDL-16)	0.2	5 (UDL-12)	5 (UDL-13)	0.6	6 (UDL-32)	0.6
IL-6 (pg/ml)	260 (37-377)	107 (14-361)	0.04*	190 (19-443)	0.5	538 (116-865)	233 (33-454)	0.01*	371 (32-676)	0.1
IL-8 (pg/ml)	218 (34-614)	90 (29-487)	0.04*	163 (30-625)	0.7	666 (150-1195)	339 (55-552)	0.001*	525 (50-882)	0.2
IL-10 (pg/ml)	240 (60-864)	100 (24-550)	0.04*	227 (54-537)	0.8	751 (74-1680)	319 (25-570)	0.01*	613 (80-1180)	0.3
IL-13 (pg/ml)	6 (UDL-16)	6 (UDL-18)	0.9	6 (UDL-14)	0.9	2 (UDL-30)	5 (UDL-16)	0.5	8 (UDL-20)	0.5
IFN-y (pg/ml)	217 (30-785)	160 (17-490)	0.2	162 (25-564)	0.4	167 (42- 644)	91 (22-300)	0.1	131 (23-392)	0.7
TNF-a (pg/ml)	316 (20-655)	88 (16-475)	0.009*	254 (20-477)	0.2	214 (36-560)	76 (36-315)	0.04*	184 (26-455)	0.6

Table 2 : Secreted cytokines concentration after C. trachomatis EBs stimulation in the presence and absence of azithromycin and doxycycline in cells obtained from women with recurrent infection Group I comprised of Chlamydia positive fertile women; Group II comprised of Chlamydia positive infertile women; CT represents Chlamydia trachomatis;

^a denotes significance level between CT+Azithromycin and CT in Group I; ^b denotes significance level between CT+Doxycycline and CT in Group I; ^c denotes Cytokine concentration is denoted by median and range in parenthesis; UDL-Under detection limit

significance level between CT+Azithromycin and CT in Group II; ^d denotes significance level between CT+Doxycycline and CT in Group II; ^{*} Denotes significance level

4. Antichlamydial drugs: Sensitivity and emergence of resistance in treatment failures

Recurrent genital *Chlamydia trachomatis* infection often results in serious sequelae and it has major impact on reproductive health. In this study, our objective was to determine the drug sensitivity profile of *C.trachomatis* isolates from patients with treatment failure and recurrent infection.

The doxycycline and azithromycin drug sensitivity assay was performed on the *C*. *trachomatis* isolates using cell culture method for Minimum Inhibitory Concentration (MIC) determination. The HeLa cells infected with *C. trachomatis* isolate were incubated with different concentrations of doxycycline and azithromycin. Subsquently, inclusions were counted after staining with *C. trachomatis* MOMP specific monoclonal antibody. Percent of infections were calculated for each isolate at different drug concentrations. These isolates showed different sensitivity patterns against doxycycline and azithromycin.

Thirteen isolates (61.9%) were found to be susceptible towards azithromycin and doxycycline with the minimum inhibitory concentration (MIC) values of $\leq 0.125 \mu g/ml$, $\leq 0.25 \mu g/ml$ respectively. Eight isolates (38%) were found less susceptible to the drugs. Two of them had MICs of $8 \mu g/ml$ for both the drugs and could not be completely eradicated as observed by minimum bactericidal concentration (MBC) assay.

Decreased antibiotic susceptibility was observed in isolates obtained from recurrently infected patients towards the current first line drugs (azithromycin and doxycycline) for chlamydial infection treatment. The MIC and MBC values of two isolates was 8 μ g/ml and it was seen that its complete eradication could not occur at this higher concentration. However, the number and size of inclusion bodies were found to be decreased with increasing concentration of antibiotics as also observed in other studies. This small percentage of organisms may reflect the presence of heterotypic resistance.

S.	Isolate	Age	Diagnosis	Previous	MIC(µ	ıg/ml)	MBC		IBC	
No.	number			treatment	Azithromycin	Doxycycline	Azithr	omycin	Doxy	cycline
							4µg/ml	8µg/ml	4µg/ml	8μg/ml
1	CT222	38	Chronic cervicitis	Doxycycline	0.12	8.0	-	-	-	-
2	CT227	30	Chronic cervicitis	Azithromycin	8.0	8.0	+	+	+	+
3	CT231	40	PID	Doxycycline	1.0	2.0	-	-	-	-
4	CT232	29	Cervicitis	Doxycycline	4.0	4.0	-	-	-	-
5	CT233	32	Cervicitis	*	0.5	0.025	-	-	-	-
6	CT235	24	Infertility	Doxycycline	2.0	4.0	-	-	+	+
7	CT244	20	PID	Doxycycline	8.0	8.0	+	+	+	+
8	CT247	32	Infertility	*	2.0	2.0	+	-	-	-

Table: Susceptibility profile of C. trachomatis against doxycycline and azithromycin.

MIC Minimum inhibitory concentration

MBC Minimum bactericidal concentration

+ Inclusion bodies detected at the given concentration of antibiotic.

- Inclusion bodies were not detected at the given concentration of antibiotic.

* Status of treatment taken not clear from the history.

5. Role of iron in pathogenesis of Chlamydia trachomatis

Chlamydial modulatory effect on host for survival is majorly conferred to intracellular factors, however, extracellular or secretory factors need to be analysed for understanding complete sequence of events leading to pathogenesis. In this direction, changes in the expression of multiple proteins using two-dimensional electrophoresis (2-DE) promise to provide a powerful strategy for characterizing complete pathophysiologic processes and designing novel drug therapies.

When *C.trachomatis* infected HeLa cells were exposed to DFO, changes were observed in the expression of secretory proteins as compared to control in 2-DE gel analysis. In these experiments, all proteins were within the 1.3–1.5-fold expression range, typical of the inherent noise in the proteomics experiment. However, based on the statistical evaluation of the variation in the baseline, we chose a 2-fold cut-off for data analysis of regulated protein expression. *C.trachomatis* infection regulated 19 proteins spots as there were 4 proteins that were down-regulated and 15 protein spots that were up-regulated more than 2-fold when cells were exposed to DFO in selected part (10-45 KDa/ 4-6) of profile. Highly abundant proteins, specifically lying between pI 4-6/below 15 kDa molecular co-ordinates were chosen for MALDI-TOF identification. Only protein spots that were down-regulated in response to the DFO treatment were excised from the gel and digested for mass spectrometry analysis. The protein within the detection range of the 12.5% polyacrylamide gels was identified as Trx-1 (pI 5.4/12.1 kDa) (Fig. 1).



Fig. 1 : Secretory protein profile of C.trachomatis infected and DFO treated C.trachomatis infected cells

6. Role of Chlamydia pneumoniae in Coronary Artery Disease (CAD) patients

Chlamydia pneumoniae heat shock protein (HSP) 60 is abundantly produced during chronic chlamydial infection and may stimulate and activate innate immune and inflammatory responses thereby contributing to atherogenesis. However, to date there is no study in which signaling cascade have been delineated in human atheromatous plaques of *C. pneumoniae* positive coronary artery disease (CAD) patients which is required for understanding *C. pneumoniae* mediated immuno-pathogenesis. Hence our aim was to study cHSP60 mediated signaling cascade in CAD patients and for this, we performed experiments at RNA and protein level in cHSP60 positive and negative group of CAD patients.

Real time PCR experiments were performed for gene expression studies- i) individual gene expression (11 genes) and ii) pathway-focused gene expression profiling (signal transduction pathway - 84 genes and MAPKinase signaling pathway - 84 genes) at RNA level. Significantly higher expression (p<0.001) was found for IL-4, IL-6, TLR-2, TLR-4, and TGF- β whereas IL-10 was found to be significantly lower (p<0.001) in cHSP60 positive CAD patients compared to cHSP60 negative. Additionally, expression of GM-CSF was higher and IFN- γ was lower in cHSP60 CAD patients.

a) Signal transduction related genes in cHSP60 positive and negative CAD patients

In our study VCAM1, NF-kB1, IL-8, ICAM1, FASLG, EGR1, BAX, and MMP7 were significantly (p<0.001) up-regulated while c-JUN, IkkB, BIRC1 were significantly (p<0.001) down-regulated in cHSP60 positive CAD patients compared to cHSP60 negative CAD patients. Moreover, expression of MDM2, IL-4, and FAS were also higher but not significant (Fig. 1).

b) MAPKinase related genes in cHSP60 positive and negative CAD patients

CREBP, CDK2, CDK4, MAPK1, MAPK8IP2, EGR1, MAPK2K6, RAC1, and EGFR were significantly (p<0.001) upregulated, however, SMAD4, MAPK9, CDKN20, MAPK10, MAPK11, ETS1, and BRAF were significantly downregulated (p<0.001) in cHSP60 positive CAD patients compared to cHSP60 negative CAD patients. Additionally, most of the ERK precursors were upregulated while; precursors of JNK and p38 were downregulated (Fig. 2).



IL-8, NF-KB1, ICAM1, VCAM1, BAX, FASLG, IL-4, IL-2, HSF1 JUN, BCL21, BIRC2, FOXA2, SELE, FOS, BRCA1, IKBKB

Fig. 1: Signal transduction pathway genes in cHSP60 positive and negative coronary artery disease patients



CREBP, CDK2, CDK4, MAPK1, MAPK8IP2, EGR1, MAPK2K6, RAC1, EGFR SMAD4, MAPK9, CDKN20, MAPK10, MAPK11, ETS1, BRAF, ELK-1

Fig. 2: MAPKinase signaling pathway genes in cHSP60 positive and negative coronary artery disease patients

7. Inclusion membrane proteins and their role in chlamydial pathogenesis

Chlamydial Inclusion membrane proteins (Incs), are involved in biochemical interactions with host cells and infecting chlamydiae. We have previously reported the role of two *Chlamydia trachomatis* (CT) Incs, namely IncB and IncC in generating host immunity in CT infected women. Emerging data shows involvement of Inc stimulated CD4 positive T-cells in aiding host immunity in infected fertile and infertile women through the secretion of interferon gamma. However the lack of data on the intra-cytokine interplay to these Incs in infected cell milieu prompted us to investigate further.

CD8 depleted, CD4 enriched cervical cells were isolated and upon stimulation with IncB and IncC, modulation of cytokines and T-cell lineage regulating transcription factors T-Bet and GATA3 was determined by real-time reverse-transcriptase (RT)-PCR and ELISA.

Significant higher expression (P < 0.05) of interferon-gamma, IL-12, IL-23 and GM-CSF were found in Inc-stimulated CD4 enriched cervical cells of CT-positive fertile women and contrastingly high IL-1 Beta, IL-4, IL-5, IL-6 and IL-10 levels were found in CT-positive infertile women. Positive correlation (P < 0.05) was found between interferon-gamma and T-Bet levels in CT-positive fertile women and IL-4 mRNA and GATA3 levels in CT-positive infertile patients upon IncB and IncC stimulation.

Our study also suggests that Incs are able to modulate expression of T-cell lineage determinants indicating their involvement in regulation of immune cells.



mRNA expression of IL-1 β , IL-4, IL-5, IL-6, IL-10, I in vitro stimulation with IncB, IncC and CT EB CSF,

Fig. 1: mRNA expression of IL-1 β , IL-4, IL-5, IL-6, IL-10, IL-12, TNF-a, IFN- γ , GM-CSF, T-Bet and GATA3 in CD4+ cervical cells in GI, GII and GIII after in vitro stimulation with IncB, IncC and CT EB. Real-time RT-PCR analysis of mRNA levels was done at 12 hours post infection where, Group I (GI) comprised of healthy women with no CT infection, Group II (GII) comprised of CTpositive fertile women, Group III (GIII) comprised of CT-positive infertile women. * P < 0.05Expression of cytokine mRNA in GII compared to corresponding levels in GI and GIII by Kruskal Wallis test. $\Delta P < 0.05$ Expression of cytokine mRNA in GIII compared to corresponding levels in GI and GII by Kruskal Wallis test.

LEISHMANIASIS

1. Identification of a novel ubiquitin-like system in the protozoan parasite Leishmania donovani

Our studies have established the presence of a Ufm1-Uba5-Ufc1 ubiquination like pathway in trypanosomatids. The cloning and characterization of LdUba5 (Ufm1 activating enzyme 5) showed homology with human Uba. Cys²¹⁷ was demonstrated as the active site Cys residue by over expressing mutated Uba5 (Cys²¹⁷ to Ser/Ala) in L. donovani cells. Further, we identified a Leishmania ubiquitin-fold modifier 1 (LdUfm1) with an exposed C-terminal glycine which is essential for subsequent activation by its cognate E1 protein (LdUba5). In *vitro* activation assay revealed the formation of a high-energy thiolester bond between LdUba5 and Ufm1 in the presence of ATP. We next investigated in Leishmania, Ufm1 conjugating enzyme (Ufc-1) which acts as an E2-like conjugating enzyme in the human Ufm1 pathway. Immuno-precipitation followed by immuno-blotting analysis showed LdUfm1 is subsequently transferred from LdUba5 to its cognate E2-like enzyme (LdUfc1) via a similar thiolester linkage with a conserved cysteine at the E2 active site. Localization studies revealed that LdUba5/LdUfm1/LdUfc1 were localized in mitochondria. Mass spectrophotometric experiments further confirmed the interaction of LdUfm1 with LdUba5 and LdUfc1. Functional role of LdUba5 activity in Leishmania growth was evident since overexpression of mutants of LdUba5 (Cys²¹⁷ to Ser/Ala) resulted in reduction of the parasite growth, indicating the importance of LdUba5 activity in Leishmania growth (Fig. 1). The unique feature of Leishmania Ufm1 pathway was its localization in the mitochondria as human Ufm1 pathway is found in cytoplasm. As LdUba5 is functionally important for the growth of Leishmania, it is possible this pathway may be involved in both protein turnover and /or transcriptional regulation like ubiquitin and other Ubls.



Fig. 1: Effect of the overexpression of LdUba5 and its mutants on the growth of the parasite

Growth of *L. donovani* promastigote cultures-plasmid control pKSNeo (WT), wild type *Ld*Uba5 and mutant *Ld*Uba5 (C>S and C>A) was monitored. Results are the mean of three independent experiments. Error bars indicate the standard deviation.

2. Increased expression of histones, PSA-2 and MAPK in natural antimony resistant Leishmania donovani

Resistance to Sodium Antimony Gluconate (SAG) is a major cause of failure to therapy in about 65% cases of visceral leishmaniasis in India. Resistance mechanisms are partly known in laboratory isolates; however the mechanisms operating in field isolates remain largely unknown. We attempted to understand the mechanism of natural antimony resistance using field isolates from kala-azar. We analysed the expression of nine genes in natural SAG resistant (n=10) and sensitive (n=4) field isolates of *Leishmania donovani* by real time PCR, in comparison with one lab-generated resistant and one standard sensitive strain (Fig. 2). The genes, MRPA, GSH-1 and AQP-1, well recognized as antimony resistant determinants on the basis of laboratory generated resistance isolates, showed expected expression level in a substantial fraction of the field isolates but not in all. We observed high expression of PSA-2 and MAPK1 in 10/10, H1 in 9/10, H2A in 8/10, H4 in 7/10 (Fig. 1) and HSP83 in 6/10 resistant isolates. Expression of PSA- 2, HSP83 and histones H1, H2A and H4 was low in all sensitive isolates while MAPK1 showed high expression in one sensitive isolate. Of these, PSA-2 and H2A genes showed a strong correlation of expression with drug susceptibility. Further, these genes showed increased expression at protein level in all resistant isolates. The data indicates that antimony resistance in field isolates is a multifactorial phenomenon. The high correlation of PSA-2 and H2A gene expression with antimony susceptibility makes them attractive candidates as biomarkers for distinguishing the resistant and sensitive parasite in the endemic area.



Fig. 2: Expression pattern of various drug resistance associated genes in L. donovani field isolates (Resistant-red, Sensitive-Green colour). Gene expression was analysed using Real Time PCR and represented as expression index with respect to LdAG83. Values given are mean \pm SD of three different experiments.

3. Studies on natural susceptibility of Indian Leishmania donovani field isolates towards paromomycin and sitamaquine and elucidation of their mechanism of action

We have established the baseline data on natural susceptibility of Indian *L. donovani* isolates towards new upcoming antileishmanial drugs, Paromomycin (PMM) and Sitamaquine (SIT) which are currently under Phase IV/III trials respectively. The data highlights the potential of PMM as an alternative anti-leishmanial drug with similar sensitivities in parasites from zones of low resistance (LR) and high resistance (HR) to antimony and showing no cross resistance towards SAG and miltefosine resistant *L.donovani* parasites. It is important to

understand the mechanism of action of these drugs. We therefore investigated how these drugs mount their cytotoxic effect on the intracellular parasites by evaluating their effect on the release of Nitric Oxide (NO) from uninfected/ *L. donovani* infected macrophages *in vitro*. There was an exponential increase in NO levels upon SIT and PMM treatment from both uninfected and *L. donovani* infected macrophages. However, the increase was higher in infected cells compared to uninfected. Despite the induction of only a small increase in the levels of NO, SIT and PMM treatment resulted in a pronounced decrease in the number of amastigotes in infected macrophages. The percentage parasite killing correlated significantly with the nitric oxide release (r=0.76, p<0.05) for PMM and SIT (r=0.48, p<0.05) treated macrophages. For SIT, upon addition of NO inhibitor, there was a decrease in NO production with decrease in the parasite killing suggesting that cytotoxic activity of SIT against *L. donovani* amastigotes may involve NO pathway. In case of PMM, there was very little effect on the parasite killing in the presence of NO inhibitor suggesting that activity of PMM against *L. donovani* may not be solely dependent on nitric oxide.

4. Association of parasitic load with interleukin-4 response in patients with cutaneous leishmaniasis due to Leishmania tropica

We have established the association between parasite burden and localized immune response in patients of Cutaneous Leishmaniasis (CL) caused by *Leishmania tropica*. Real-time PCR was employed to measure parasitemia in tissue lesions of CL patients at pre-treatment (n=26) and post-treatment stage (n=10). *L. tropica* was detected in all CL lesions with a mean value of 1,18,357 parasites/g of dermal tissue. Following treatment, only 1/10 patient showed residual parasites (100 parasite/g tissue). Parasite load was high (mean, 3,06,000 parasite/g tissue) in acute infections (early lesions) and low (mean, 1081 parasite/g tissue) in chronic infections (late lesions). Intra-lesional transcripts of interferon- γ , tumor necrosis factor- α , interleukin (IL)-1 β , IL-8, IL-10 and IL-4 were investigated in early lesions (≤ 2 months, n=14) and late lesions (>2 months, n=15) by reverse transcriptase-PCR, where IL-4 was found significantly up-regulated in early lesions (p<0.02). Further, levels of parasite burden and IL-4 were distinctly correlated in various clinical forms of CL (Fig. 3). Other cytokines were at comparable levels in early/ late lesions and in different clinical forms. Up-regulation of IL-4 was correlated with higher parasite burden in early lesions of CL, which may be involved in pathogenesis of CL by inhibiting protective immune response.



Fig. 3: Comparative assessment of intra-lesional interleukin [IL]-4 mRNA expression in patients with CL. A. Level of IL-4 in early lesions (≤ 3 months, n=14) and in late lesions (≥ 4 months, n=15). B. Level of IL-4 in various polymorphic forms of CL; erythematous plaque (EP, n=5), ulcerated plaque (UP, n=3) and granulomatous nodule (GN, n=3) erythematous ulcerated (EU, n=15), erythematous nodule (EN, n=5)

ADULT STEM CELL BIOLOGY

1. Optimal attenuation conditions for 3T3 fibroblasts for use as feeder cells

The adult and embryonic stem cells are effectively established *in vitro* using feeder cells that are growth arrested by either gamma irradiation or exposure to mitomycin C. However, an abrupt overgrowth of feeders as a consequence of failed attenuation while using mitomycin C is a serious setback. Since mitomycin C approach is convenient and does not require high establishment and operational costs as with gamma-irradiation, the same has been pursued to grow Cultured Epithelial Autografts (CEA). Earlier it was shown that mitomycin C was effective as an attenuating agent on 3T3 fibroblasts in a 'numerical' dose-dependant manner. Subsequent experiments carried out indicated that such differentially growth arrested feeders proportionately stimulated the proliferation of human epidermal keratinocyte stem cells. The study is continued to evaluate the effectiveness of various numerical doses of mitomycin C in the medium on 3T3 fibroblast in stimulating human epidermal keratinocyte stem cells.

The successful experimental evidences in the direction of identifying a fool-proof method of growing human epidermal keratinocyte stem cells were filed as a **patent**. In this patent description, it was shown that differentially attenuated feeders produced by titrating with arithmetically derived numerical doses of mitomycin C brought about irreversible attenuation and increase in keratinocyte cell proliferation in a dose dependent manner as revealed by differential counts in a co-culture system performed during 9-days period.

The BrdU incorporation studies undertaken in continuation, revealed a significantly (P<0.01) higher proliferation index in those keratinocyte cultures grown in presence of an effective & irreversible feeders compared to less effective or reversible feeders (Fig. 1). The reversal of growth arrest finally caused contamination of keratinocytes consequently ending in stunted growth of keratinoctytes with proliferating 3T3 cells.



Fig. 1: Human epidermal keratinocytes stained with anti-BrdU antibody after they were grown in presence of feeders from effective feeders & irreversible (left), less effective & irreversible feeders (middle) and reversible feeders (right) in which feeder cells are persistent.

The results are concomitant with keratinocyte-3T3 differential cell count experiments. In continuation, research work towards characterization of CEA to be produced by employing the innovative method on a more stable feeder cell line will be initiated as part of the newly sanctioned project entitled "A novel arithmetic approach for fool-proof production of growth arrest in 3T3 cells suitable for human epidermal culture".

2. Investigation into the utility of a patented synthetic thermo-reversible hydrogel polymer as supportive matrix towards the development of 3-D composite skin for application in wound healing and other dermatological disorders

Tissue engineering involving *in vitro* construction of 3-dimensional cellular structures using various matrix materials is one of the recent approaches for wound healing. A synthetic non-toxic thermoreversible gelation polymer hydrogel, Mebiol gel, has been proven to support hepatic progenietor cells, corneal limbal cells and several other cell lines. Mebiol gel is being tested to support the growth and differentiation of epidermal keratinocyte stem cells which could pave way to their large scale expansion under xenogeneic free culture conditions.

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

Previously it has been shown that a keratinocyte pellet sandwiched (Fig. 2, left) in between two discs of this patented Mebiol gel without feeders, yielded results suggestive of proliferation and migration of a clone of keratinocytes predominantly of smaller size of about $9\mu m$ (Fig. 2, right).



Fig. 2: Schematic diagram representing sandwich model of Mebiol gel with epidermal keratinocytes (left) and a plume of migratory keratinocytes emerging from the pellet of cells (right).

Efforts to isolate these cells have been futile so far largely because of the thermally sensitive nature of the gel resulting in mixing up of all cell populations. A direct approach has been planned presuming that this exclusive clone of cells is likely to be the population of Human Epidermal Keratinocyte Stem Cells (HEKSCs) that have been recently shown (Juxue et al. 2008, *Cell Research* 18:360–371) to possess specific characteristics like P63, PCNA and β 1-integrin positivity, rapid adhesiveness to collagen type IV and higher colony forming efficiency and form differentiated epidermis using the conventional Rheinwald and Green culture system. Accordingly, a strategy to first isolate and enrich the pure and viable HEKSCs based on collagen type IV adhesiveness followed by plating them in the sandwich model of Mebiol gel has been adopted. Initially a large population of keratinocytes (5 x 10⁶) was grown followed by their isolation based on their property of time-lag in adhesiveness on collagen type IV. Cells that have attached within 20 minutes (RA cells), overnight (SA cells) and neveradhering (NA) cells were collected, smeared over cover-glasses and air-dried. These isolated populations are now being tested for their specific markers.

The work is in progress and the outcome of this approach depends on the ability of RA, SA and NA cells to exhibit differential turnover rates in Mebiol gel-sandwich model. This process will enable identification of the proliferating clone of cells and pave way to establish an innovative process to enrich HEKSCs.

ENVIRONMENTAL TOXICOLOGY

Human beings are exposed to a large scale of pollution resulting from a variety of manmade factors. The effects of such pollution are evident from a wide range of human healthrelated problems. While there are several reports of toxic effects of pollutants on living beings, the majority of these remain confined to segregated animal studies possibly linking to health effects in human beings. To our knowledge, there are only a few reports directly linking the presence of pollutants in human body and the problems being faced by man.

1. Health hazards of phthalate vis-à-vis idiopathic male infertility

Phthalates are di-esters of phthalic acid (1,2-benzenedicarboxylic acid). These are a group of man-made chemicals with a wide spectrum of industrial applications such as personal and car care products, dyes, vinyl flooring, adhesives and sealants, toys, food packaging, blood storage bags and medical devices. Phthalates are fat soluble and can be absorbed through the skin, inhaled as fumes and ingested when they contaminate food. The obvious relationship of endocrine and reproductive toxicities in humans with the exposure to phthalate has not been described in the literature. This project has been planned to investigate effects of phthalate exposure on human male infertility.

A total of 125 patients, who came for infertility treatment to Urology OPD of Safdarjang Hospital, New Delhi were prospectively enrolled for this study. Twenty healthy fertile men whose partners had a time-to pregnancy (TTP) of \leq 12 months were enrolled as controls. Based on occupation, the patients as well as controls were divided into 3 groups, viz.: high, medium and low risk.

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

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

Low risk group: Individuals reporting minimum exposure to phthalates at home or work.

The sperm count was more than 20 million per millilitre of semen in all the controls. All the control samples showed normal motility and morphology. Thirty one percent of low risk group patients, 30% of medium risk group and 46% of high risk group patients were azoospermic. Analysis of remaining samples of patients revealed normal sperm count in only 41%, 35% and 29% of low, medium and high risk groups respectively. The sperm motility was below reference range in 54%, 31%, 57% of low, medium and high risk groups respectively.

Mean testosterone levels were lower in patient group $(5.8\pm2.2 \text{ ng/ml})$ as compared to controls $(6.0\pm1.66 \text{ ng/ml})$. The decrease was more significant in medium and high risk groups (mean 4.9 ng/ml). In contrast, the estradiol levels were elevated in patients (mean 14.6 pg/ml) in comparison to healthy controls (mean 6.2 pg/ml). With the increasing potential of occupational phthalate exposure in high risk groups, the estradiol levels varied from 4.4 to 57.7 pg/ml (mean 15.7 pg/ml).

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

Estimation of phthalates by Gas Chromatography revealed presence of one or more phthalate in 74% of infertile patients as compared to 47.6% of control subjects. All the patients of high risk infertile group exhibited occurrence of 2 to 14 phthalates in comparison to only one phthalate observed in only one sample of high risk controls. The study is being continued.

2. Assessment of pesticide exposure in tea garden workers of northeastern state of India (HEBM)

India is one of the major tea producing countries with 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. Farmers and agricultural workers are exposed to pesticides directly when they are mixing and spraying these pesticides, especially so in developing countries such as Asia. Every year, about 3 million people are poisoned around the world and 200,000 die from pesticide.

Biomonitoring is a scientific technique for assessing human exposures to natural and synthetic chemicals, based on sampling and analysis of an individual's tissues and fluids. While blood, urine, breast milk and expelled air are most commonly measured; hair, nails, fat, bone and other tissues may also be sampled. Sriramachari et al described the role of human placenta for biomonitoring of toxic trace metals and pesticides. Present study has been designed to assess the pesticide exposure of tea garden workers in north-eastern states of India in placenta and blood of women.

So far, 164 samples of placenta, cord blood and maternal blood have been collected from Assam Medical College, Dibrugarh which included 95 tea garden workers, 25 non-tea garden agricultural workers and 44 controls. Similarly 65 respective samples were collected from Safdarjung hospital. The samples of placenta as well as blood were processed for homogenization and extraction of organic pollutants. Simultaneously, a cocktail of commonly used organochlorine and organophosphorus pesticides and fungicides used in agriculture and for domestic purpose was selected for multi-residue analysis. After a large number of repeated trials, the analytical conditions for reversed phase HPLC (*Shimadzu Model* LC-20AD) were optimized and standardized so as to distinctly separate the selected pollutants.

Initial investigations have revealed presence of several pesticides such as malathion, endosulfan, fenvalerate, dimethoate, tebuconazole, δ -BHC, ethion, atrazine, chlorpyriphos, quinalphos, flufenacet, DDT, pyrethroids, glyphosate, paraquat, dicofol, monocrotophos, etc. and PAH, viz.: pyrene, naphthalene and dibenzoanthracene in the samples analyzed so far, thereby demonstrating that human placenta can be used for comprehensive HEBM. Analysis of remaining samples is in progress.

MAJOR ACTIVITIES AT INSTITUTE OF PATHOLOGY

- Dr. VM Katoch, Secretary, Department of Health Research & Director General (ICMR) delivered the 9th Smt. Pushpa Sriramachari Founder's Day Oration on 'Understanding of granulomas with special reference to mycobacterial diseases' on 18th January 2010.
- Professor Indira Nath, Ramanna Fellow & Dr. Sunita Saxena, Director, IOP, New Delhi organized 'International Workshop on Molecular & GIS Based Epidemiology of Leprosy' at Institute of Pathology (ICMR) from 4th 9th March 2010.



• On the eve of 15th August 2009 and 26^{th} January 2010, flag hoisting was done at IOP.



IOP Library conducted Workshop and Training Programme on JCCC@ICMR ERMED Consortia through Informatics India on 4th September 2009.



Scientific Advisory Committee Meeting was held at IOP on 13th October 2009.



Organized quarterly meeting of Delhi chapter of Indian Association of Pathologists and Microbiologists on 5th December 2009. Dr. Avninder Pal Singh, Scientist 'C', IOP gave a talk on "Tissue microarray as a tool in validation and discovery of tumor markers".





Dr. Anavaj Sakuntabai, Institut Pasteur, Paris gave a seminar at IOP in the framework of Bonjour-India, Festival of France on 16th December 2009.



 Guest lecture by Dr. Prakash Hrideyesh,, German Cancer Research Centre, Heidelberg, Germany, on "The Macrophages : Potential Target for translational Medicine" on 7th January 2010.



AWARDS AND HONOURS

1. **Dr. Sunita Saxena**, Director, IOP was awarded the **Novartis Oration Award** for research in the field of Cancer by ICMR for the year 2006 on 18th Sept. 2009.



2. **Dr. Aruna Mittal**, Dy. Director (Sr. Grade) was awarded **ICMR Lala Ram Chand Kandhari** Award for research in the field of *Chlamydia trachomatis* by ICMR for the year 2007 on 18th Sept. 2009.



PATENT

Yerneni LK and Kumar A: A culture system for the growth of stem cells. Indian Patent File No. 2086/DEL/2009.

EXTRAMURAL PROJECTS

NEW PROJECTS

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

ONGOING PROJECTS

- Characterisation of host immune factors associated with progression of superficial TCC of bladder by microarray analysis.
 Dr. Sunita Saxena (2008-11).
- Establishment of breast cancer cell lines from primary breast tumours.
 Dr. Sunita Saxena, Dr. Sujala Kapur, Dr. Usha Agrawal DBT (2008-11).
- 3. Study on gene expression and hypermethylation profiles in early onset breast cancer. Dr. Sunita Saxena, Dr. Sujala Kapur, Dr. BSA Raju - ICMR Multicentric Task Force Project (2007-10).
- 4. Role of tobacco use in causation of cancer in north-east India. Dr. Sunita Saxena, Dr. Sujala Kapur, Dr. Usha Agrawal - ICMR Multicentric Task Force Project (2005-10).
- 5. Effect of pesticide exposure in causation of cancer in north-east India. Dr. Sunita Saxena, Dr. Sujala Kapur, Dr. Usha Agrawal - ICMR Multicentric Task Force Project (2005-10).
- New tools for monitoring drug resistance and treatment response in visceral leishmaniasis in the Indian subcontinent.
 Dr. Poonam Salotra European Commission (2009-12).
- 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).
- 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).

- 9. Transcriptome profiling for identification and characterization of miltefosine resistance associated genes of *Leishmania donovani*.
 Dr. Poonam Salotra ICMR (2009-12).
- Analysis of host immuno-determinants involved in the pathogenesis of Indian cutaneous leishmaniasis exploiting cDNA microarray.
 Dr. Poonam Salotra ICMR (2007-10).
- 11. Assessment of pesticide exposure in tea garden workers of north-eastern state of India (HEBM).
 Dr. AK Jain ICMR (2008-10).
- Investigation into the utility of a patented synthetic thermo-reversible hydrogel polymer as supportive matrix towards the development of 3-D composite skin for application in wound healing and other dermatological disorders.
 Dr. LK Yerneni - ICMR (2007-10).

COMPLETED PROJECTS

- Microsatelite instability in androgen receptor gene, p53 gene polymorphisms/mutations and expression profile of mismatch repair genes in prostate cancer.
 Dr. Sunita Saxena - DST (2005-08).
- Comprehensive study of carcinoma oesophagus at northeast India Multidiscipline approach.
 Dr. Sunita Saxena, Dr. Sujala Kapur ICMR Multicentric Task Force Project (2004-08).
- Evaluation of host immunodeterminants involved in the pathogenesis of kala-azar and post kala-azar dermal leishmaniasis using cDNA array.
 Dr. Poonam Salotra DRDO (2006-09).

PUBLICATIONS

- 1. Chintamani Chintamani, Khandelwal Rohan, Tandon Megha, K Yashwant, Kulshreshtha Pranjal, Aeron Tushar, Bhatnagar Dinesh, Bansal Anju, Saxena Sunita. Carcinoma developing in a fibroadenoma in a woman with a family history of breast cancer: A case report and review of literature. **Cases Journal**, 2: 9348, 2009.
- 2. Chattopadhyay I, Singh A, Phukan R, Purkayastha J, Kataki A, Mahanta J, Saxena S, Kapur S. Genome-wide analysis of chromosomal alterations in patients with esophageal squamous cell carcinoma exposed to tobacco and betel quid from high-risk area in India. **Mutation Research Genetic Toxicology and Environmental Mutagenesis**, 696: 130-138, 2010. DOI information: 10.1016/j.mrgentox.2010.01.001
- 3. Chattopadhyay I, Phukan R, Singh A, Vasudevan M, Purkayastha J, Hewitt S, Kataki A, Mahanta J, Kapur S, Saxena S. Molecular profiling to identify molecular mechanism in esophageal cancer with familial clustering. **Oncol Rep**, 21(5): 1135-1146, 2009. PMID: 19360286.
- 4. Singh Avninder, Kapur Sujala, Chattopadhyay I, Purkayastha Joydeep, Sharma Jagannath, Mishra Ashwani, Hewitt M Stephen, Saxena Sunita. Cytokeratin immunoexpression in esophageal squamous cell carcinoma of high-risk population in northeast India. Applied Immunohistochemistry & Molecular Morphology, 17(5): 419-424, 2009. PMID: 19417629.
- 5. Kaushal Mishi, Chattopadhyay Indranil, Phukan Rupkumar, Purkayastha Joydeep, Mahanta Jagadish, Kapur Sujala, Saxena Sunita. Contribution of germline BRCA2 sequence alterations to risk of familial esophageal cancer in high-risk area of India. **Disease of the Esophagus**, 23: 71-75, 2010.
- 6. Thoudam Regina Devi, Yadav Dhirendra Singh, Mishra AK, Ihsan Rakhshan, Kaushal Mishi, Chattopadhyay Indranil, Chauhan Pradeep Singh, Sharma Jagannath, Zomawia Eric, Verma Yogesh, Nandkumar A, Mahanta Jagadish, Phukan Rupkumar, Kapur Sujala, Saxena Sunita. Distribution of GSTT1 and GSTM1 polymorphisms in north east Indians. **Genet Test Mol Biomarkers**, 14(2): 163-9, 2010.
- 7. Chakraborty Anurupa, Mishra AK, Soni Abha, Regina Thodum, Mohil R, Bhatnagar D, Bhatnagar A, Chintamani Chintamani, Sharma PC, Saxena Sunita. Vitamin D receptor gene polymorphism(s) and breast cancer risk in north Indians. **Cancer Detection and Prevention**, 32: 386-394, 2009.
- 8. Agrawal T, Gupta R, Dutta R, Srivastava, Bhengraj PR, Salhan S, Mittal A. Protective or pathogenic immune response to genital chlamydial infection in women-A possible role of cytokine secretion profile of cervical mucosal cells. **Clinical Immunology**, 130: 347-354, 2009.

- 9. Srivastava P, Jha HC, Salhan S, Mittal A. Azithomycin treatment modulates cytokine production in *Chlamydia trachomatis* infected women. **Basic and Clinical Pharmacology & Toxicology**, 104 : 478-482, 2009.
- 10. Agrawal T, Vats V, Salhan S, Mittal A. Determination of chlamydial infectious load and immune parameters in asymptomatic, symptomatic and infertile women. **FEMS**, 55: 250-257, 2009.
- 11. Jha H, Prasad J, Mittal A. Association of plasma circulatory markers, *Chlamydia pneumoniae* and high sensitive C-reactive protein. **Mediators of Inflammation**, 561532, 2009.
- 12. Gupta R, Salhan S, Mittal A. Seroprevalance of antibodies against *C. trachomatis* inclusion protein B & C in infected women. Jour of Infection in Developing Countries, 3: 3, 2009.
- 13. Jha R, Bas S, Salhan S, Mittal A. In infertile women, cervical epithelial cells from *Chlamydia trachomatis* infected site co-express higher level of chlamydial heat shock protein 60 and 10 than in fertile women. **Gynaecologic and Obstetric Investigation**, 68: 160-166, 2009.
- 14. Gupta R, Salhan S, Mittal A. Host immune responses to chlamydial inclusion membrane proteins B and C in *Chlamydia trachomatis* infected women with or without fertility disorders. **Reproductive Biology & Endocrinology**, 7: 38, 2009.
- 15. Vardhan H, Dutta R, Vats V, Gupta R, Jha H, Jha R, Srivastava P, Bhengraj A, Mittal A. Persistently elevated level of IL-8 in *Chlamydia trachomatis* infected HeLa 229 cells is dependent on intracellular available iron. **Mediators of Inflammation**, 2009. doi:10.1155/2009/417658.
- 16. Vardhan H, Bhengraj A, Jha R, Mittal A. *Chlamydia trachomatis* alters iron regulatory protein-1 binding capacity and modulates cellular iron homeostasis in Hela-229 cells. **Jour Biomed Biotech**, 2009. doi:10.1155/2009/342032.
- 17. Sachdeva P, Patel A, Sachdev D, Ali, Mittal A, Saluja, D. Evaluation of indigenous PCR assay for detection of *Chlamydia trachomatis* using direct fluorescent assay and Roche amplicor detection kit. **Jour Med Microbiol**, 58: 867-873, 2009.
- 18. Jha H, Srivastava P, Prasad, Mittal A. Evaluation of plasma circulatory markers, *Chlamydia pneumoniae* and high sensitive C-reactive protein in coronary artery disease patients. Atherosclerosis Supplements, 10, 2009.
- 19. Jha H, Srivastava P, Prasad J, Mittal A. Coronary artery disease: Biomarkers in first degree relatives. Atherosclerosis Supplements, 10, 2009.

- 20. Gupta R, Salhan S, Mittal A. Modulation of cytokines and transcription factors (T Bet and GATA3)) in CD4 enriched cervical cells of *Chlamydia trachomatis* infected fertile and infertile women upon stimulation with chlamydial inclusion membrane proteins B and C. **Reproductive Biology & Endocrinology**, 7: 84, 2009.
- 21. Jha H, Prasad J, Mittal A. The plasma circulatory markers in male and female coronary artery disease patients. **Heart and Lung, 2009, DOI 10.1016/j.hrtlng.2009.10.005**
- 22. Jha H, Prasad J, Mittal A. Prevalence of *Chlamydophila pneumoniae* is higher in aorta than in carotid artery in coronary artery disease patients. Acta Pathologica Microbiol Immunol Scan, 117: 905-11, 2009.
- 23. Bhushan B, Chauhan PS, Saluja S, Verma S, Mishra AK, Siddiqui S, Kapur S. Aberrant phenotypes in childhood and adult acute leukemia and its association with adverse prognostic factors and clinical outcome **Clin Exp Med**, 10(1): 33-40, 2010.
- 24. Chauhan Pradeep S, Bhushan Bharat, Mishra AK, Singh LC, Saluja Sumita, Verma Saurabh, Gupta Dipendra K, Mittal Vishakha, Chaudhry Sumita, Kapur Sujala. Mutation of FLT3 gene in acute myeloid leukemia with normal cytogenetics and its association with clinical and immunophenotypic features. **Medical Oncology**, 31, 2010.
- 25. Das RP, Jain AK, Ramesh V. Current concepts in pathogenesis of psoriasis. Ind J Dermatol, 54: 7-12, 2009.
- 26. Verma S, Kumar R, Katara GK, Singh LC, Negi NS, Ramesh V, Salotra P. Quantification of parasite load in clinical samples of leishmaniasis patients: IL-10 level correlates with parasite load in visceral leishmaniasis. **PLoS One**, 5(4): e10107, 2010.
- 27. Ramesh V, Kumar J, Kumar D, Salotra P. A retrospective study of intravenous sodium stibogluconate alone and in combination with allopurinol, rifampicin, and an immunomodulator in the treatment of Indian post-kala-azar dermal leishmaniasis. **Ind J Dermatol Venereol Leprol**, 76(2): 138-44, 2010.
- 28. Kumar D, Ramesh V, Verma S, Ramam M, Salotra P. Post-kala-azar dermal leishmaniasis (PKDL) developing after treatment of visceral leishmaniasis with amphotericin B and miltefosine. **Ann Trop Med Parasitol**, 103: 727-730, 2009.
- 29. Sharma P, Gurumurthy S, Duncan R, Nakhasi HL, Salotra P. Comparative *in vivo* expression of amastigote up regulated *Leishmania* genes in three different forms of leishmaniasis. **Parasitol Int**, 59: 262-264, 2010.
- 30. Kumar R, Bumb RA, Salotra P. Evaluation of localized and systemic immune responses in cutaneous leishmaniasis caused by *Leishmania tropica*: Interleukin-8, monocyte chemotactic protein-1 and nitric oxide are major regulatory factors. **Immunology**, 130: 193-201, 2010.

- 31. Kumar R, Bumb RA, Salotra P. Correlation of parasitic load with interleukin-4 response in patients with cutaneous leishmaniasis due to *Leishmania tropica*. FEMS **Immunol Med Microbiol**. 57(3): 239-46, 2009.
- 32. Agrawal Usha, Sharma Monika, Bhatnagar Dinesh, Saxena Sunita. Leydig cell tumor An unusual presentation. Ind J of Path and Micro, 52(3),2009.
- 33. Chintamani, Aeron T, Mittal M, Bhatnagar D, Agarwal U, Saxena S. Are the structures preserved in functional neck dissections truly preserved functionally? A prospective study of patients with head and neck cancer at a tertiary cancer care center. Oral Oncology Supplement, 3(1): 175, 2009.
- 34. Murthy NS, Agarwal Usha, Nandakumar BS, Prutvish S, Chaudhary K. Obesity and colorectal cancer. **ICMR Bulletin**, 39 (7–9), 2009.
- 35. Paliwal Purnima, Hora Harsh, Agrawal Usha. Capillary vessels in Ewing's sarcoma: Overlooked? J of Cytology, 26: 3, 2009.
- 36. Murthy NS, Chaudhry K, Nadayil D, Agarwal UK, Saxena S. Changing trends in incidence of breast cancer: Indian scenario. Ind J of Cancer, 46(1): 73-74. 2009.
- 37. Brahama Manoja Kumar, Deohare Preeti, Verma Saurabh, Ratha Srikanta Kumar, Garg Puja, Biswal Prasanta Kumar, Chowdhary Piyali Dhar, Ray Madhur. The neuronal apoptic death in global cerebral ischemia in gerbil: Important role sodium channel modulator. J Neurosci Res, 87(6): 1400-11, 2009.
- 38. Bihaqi SW, Sharma M, Singh A, Tiwari M. Neuroprotective role of Convolvulus pluricaulis on aluminium induced neurotoxicity in rat brain. J Ethnopharmacol, 124(3): 409-15, 2009.
- 39. Malhotra P, Singh A, Ramesh V. Syringocystadenoma papilliferum in the thigh. **Ind J Dermatol Venreol Leprol**, 75(2): 170-172, 2009.
- 40. Singh A, Vermani S, Shruti S. Choroid plexus carcinoma: Report of two cases. Ind J Pathol Microbiol, 52(3): 405-407, 2009.
- 41. Sarvjot V, Sharma S, Mishra S, Singh A. Melasma: A clinicopathological study of 43 cases. **Ind J Pathol Microbiol**, 52(3): 357-59, 2009.



ACCEPTED FOR PUBLICATION

- 1. Yadav Dhirendra Singh, Thoudam Regina Devi, Ihsan Rakhshan, Mishra AK, Kaushal Mishi, Chattopadhyay Indranil, Chauhan Pradeep Singh, Sharma Jagannath, Zomawia Eric, Verma Yogesh, Nandkumar A, Mahanta Jagadish, Phukan Rupkumar, Saxena Sunita, Kapur Sujala. Polymorphisms of Glutathione-S-transferase (GST) genes and the risk of aerodigestive cancers in Northeast Indian population. **Genetic Testing and Molecular Biomarker**.
- 2. Rakshan I, Chattopadhyay I, Phukan R, Mishra AK, Purkayastha J, Sharma J, Zomawia E, Verma Y, Nandkumar A, Mahanta J, Saxena S, Kapur S. Role of EPHX1 gene polymorphisms in esophageal cancer of high-risk area in India. Jr. of Gastroenterology and Hepatology.
- 3. Agrawal Usha, Mishra Ashwani K, Salgia Payal, Verma Saurabh, Mohanty Nayan K, Saxena Sunita. Role of tumor suppressor and angiogenesis markers in prediction of recurrence of non-muscle invasive bladder cancer. Pathology and Oncology Research.
- 4. Bhengraj AR, Goyel A, Talwar GP, Mittal A. Assessment of antichlamydial effects of a novel polyherbal tablet "BASANT". **Sexually transmitted infection**. 2009.
- 5. Jha H, Prasad J, Mittal A. Sequencing of *Chlamydia pneumoniae* in coronary artery disease patients attending tertiary hospital in New Delhi, India. American Jour of Inf. Control. 2009.
- 6. Vats V, Agrawal T, Salhan S, Mittal A. Characterization of apoptotic activities during *Chlamydia trachomatis* infection in primary cervical epithelial cells. **Immunological Investigations**. 2010.
- 7. Vardhan H, Bhengraj A, Jha R, Mittal, A. Higher expression of ferritin protects *Chlamydia trachomatis* infected HeLa 229 cells from reactive oxygen species mediated cell death. **Biochemistry and Cell Biology**, 2010.
- 8. Bhengraj A, Vardhan H, Srivastava P, Salhan S, Mittal A. Decreased susceptibility towards azithromycin and doxycycline in clinical isolates of *Chlamydia trachomatis* obtained from recurrently infected female patients in India. **Chemotherapy** 2010. **DOI:10.1159/000314998.**
- 9. Soni Simmi, Rath Gayatri, Prasad Chandra Prakash, Salhan Sudha, Saxena Sunita, Jain Arun Kumar. Apoptosis and Bcl-2 protein expression in human placenta over the course of normal pregnancy. **Anatomia Histologia Embryologia.**
- 10. Rath G, Jain AK, Mukherjee A, Bastia B, Shrivastava P, Raghunandan C. Placental vasculosyncytial membrane in tobacco exposed Indian mothers: A quantitative and ultrastructural study. J. Int. Med. Sci. Acad.

- 11. Agrawal Usha, Rai Himanshu, Jain Arun K. Morphological and ultrastructural characteristics of Extracellular Matrix (ECM) changes in Oral Squamous Cell Carcinoma (OSCC). Ind J of Dental Res.
- 12. Aggarwal A, Agarwal U, Verma S, Mohanty NK, Saxena S. Serum Th1 and Th2 cytokines balance in patients of superficial transitional cell carcinoma of bladder pre and post intravesicle combination therapy. **Immunopharmacol Immunotoxicol.**

ABSTRACTS/PROCEEDINGS

- Kapur Sujala, Chattopadhyay Indranil, Phukan Rupkumar, Purkayastha Joydeep, Marshal Vikki, Kataki Amal, Mohanta Jagdish, Bowtell David, Saxena Sunita. Genome-wide analysis of genetic alterations in esophageal squamous cell carcinoma by snp array. 20th Asia Specific Cancer Conference, 12th –14th November, 2009, Japan.
- 2. Saxena Sunita, Wasson Mishi Kaushal, Chatterjee Indranil, Bhatnagar A, Chintamani, Bhatnagar D, Kapur Sujala. Genome-wide analysis of DNA copy number variations in Indian breast cancer patients using high-density SNP arrays 20th Asia Specific Cancer Conference, November 12-14, 2009, Japan. (Poster)
- 3. Saxena Sunita, Kaushal Mishi, Chatterjee Indranil, Bhatnagar A, Chintamani, Bhatnagar D, Kapur Sujala. Genome-wide analysis of genetic alterations in breast cancer patients from Northeast India using 10k SNP arrays. Organisation for Oncology and Translational Research, 26th-27th Feb. 2010, Kyoto (Poster).
- 4. Chauhan Pradeep Singh, Ihsan Rakhshan, Mishra Ashwani Kumar, Bhushan Bharat, Kaushal Mishi, Yadav Dhirendra Singh, Regina Thoudam Devi, Soni Abha, Chattopadhyay Indranil, Saxena Sunita, Kapur Sujala. Glutathione S-transferase and Microsomal Epoxide Hydrolase Gene Polymorphisms and Risk of Acute myeloid leukemia. First international conference on Hematologic Malignancies: Bridging the Gap 2010 Feb 5-7, 2010, Singapore City, Singapore (Poster).
- 5. Chattopadhyay Indranil, Singh Avninder, Kapur Sujala, Saxena Sunita. Genome-wide mRNA profiling of familial and non-familial esophageal squamous cell carcinoma for identification of molecular mechanism of esophageal cancer in high-risk area of India. 2009 NCRI Cancer Conference, Birmingham, UK (Poster No.B148).
- Agarwal Shweta, Saluja Sumita, Paliwal Purnima, Jairajpuri Zeeba S, Kapur Sujala. Angiogenesis and the evidence of endothelial mesenchymal transition (EndMT) in bone marrow disorders showing fibrosis. 6th Asia Pacific Indian Association of Pathologists (IAP) Congress, 20th – 23rd August, 2009, Kochi.
- Singh Avninder, Kapur Sujala, Saxena Sunita. Tissue Microarray: A high throughput chip for the pathologist. 58th Annual Conference of IAPM, Science City, Kolkata. 18th -23rd December 2009.

- 8. DNA Microarray in Cancer: Principle, Methodology and Implications. RGCON-2010 CME, New Delhi, March 2010.
- 9. Rastogi S, Yadav C, Misro MM, Das B, Salhan S, Mittal A. *Chlamydia trachomatis* infection in spontaneous aborters. Proceedings of International Conference on Reproductive Health, Jaipur, 2010.
- 10. Chugh Rishi Man, Mori Yuichi, Yoshioka Hiroshi, Abraham Samuel, Yerneni LK Growth of human epidermal keratinocytes in Mebiol gel. Proceedings of Symposium on Regenerative Medicine, Nichi-in Centre of Regenerative Medicine (NCRM), Chennai, 24th October 2009.

SCIENTIFIC ACTIVITIES

Dr. Sunita Saxena

- 1. Attended seminar on Next Generation Sequencing held at JNU, New Delhi on 9th April 2009.
- 2. Attended IAPM (Delhi Chapter) meeting held at University of Delhi organized by U.C.M.S., Delhi on 11th April 2009.
- 3. Attended 4th Medical Development Congress on Stem Cell Research & Therapy held at Assocham House, New Delhi on 13th April 2009.
- 4. Attended SAC meeting held at Institute of Cytology & Preventive Oncology, Noida on 17th April 2009.
- 5. Attended Screening Committee meeting for the post of Scientist 'C' held in ICMR on 22^{nd} April 2009.
- 6. Attended PRC Meeting to consider proposals under Cellular and Molecular Biology held at ICMR H.Q. on 22nd April 2009.
- 7. Attended Selection Committee Meeting for the selection for the post of SRF at Dept. of Microbiology, S.J. Hospital, New Delhi on 24th April 2009.
- 8. Invited to attend the first DSMB meeting on "Curcumin Clinical Trial in Ca Cx Cancer" on 4th May 2009 at NII, New Delhi.
- 9. Attended Selection Committee Meeting, walk-in interview for the project at Dept. of Microbiology, S.J. hospital, New Delhi on 5th May 2009.
- 10. Chaired Ethical Committee Meeting held at Safdarjang hospital on 5th May 2009.
- 11. Invited to attend Ph.D. viva as a reviewer held at Dr. B.R. Ambedkar University, Agra on 8th May 2009.
- 12. Attended viva for registration for Ph.D. as a guide of Ms. P. Shanti Latha held at BITS, Pilani on 15th May 2009.
- Attended Brain Storming Session called by Secretary, Department of Health Research & Director General, ICMR, New Delhi held at ICMR on 19th May 2009.
- 14. Inspected Western Common Hospital, Chandimandir, Haryana on 20th May 2009 for renew of accreditation for conducting DNB course of NBE, New Delhi.

- 15. Invited to attend Ph.D. viva as a reviewer held at PGIMR, Chandigarh on 20th May 2009.
- 16. Attended First Symposium on HPV Vaccination in the Asia Pacific and Middle East Region held at Seoul, Korea during 1st 3rd June 2009.
- 17. Attended Task Force Project Meeting at ICMR H.Q. on 18th June 2009.
- 18. Attended Project Review Committee meeting of NCD Division, ICMR held at ICMR on 25th June 2009.
- 19. Chaired Ethical Committee meeting of Safdarjang hospital, New Delhi on 30th June 2009.
- 20. Attended Project Review Committee meeting on North-East held at ICMR on 6th July 2009.
- 21. Attended Selection Committee meeting for the selection of Scientist 'C' held at ICMR, New Delhi on 21st July 2009.
- 22. Chaired the session on "Renal Pathology" (talk given by Dr. Vinita Batra) at Delhi Chapter of IAPM held at Maulana Azad Medical College, New Delhi on 1st Aug. 2009.
- 23. Chaired Ethical Committee meeting of Safdarjang hospital, New Delhi on 6th August 2009.
- 24. Nominated to visit as an expert, Cell Biology Division, Gujrat Cancer and Research Institute, MP Shah Cancer Hospital, NCH Campus, Asarwa, Ahmedabad by NCD-ICMR on 10th August 2009.
- 25. Attended the meeting of Directors held at National Board of Examinations, New Delhi on 18th August 2009.
- Nominated by ICMR to attend ICMR University of Minnesota Workshop on Cancer & Diabetes held during 26th - 28th August 2009 at Minneapolis, University of Minnesota.
- 27. Attended Scientific Advisory Committee meeting of Institute of Pathology held at IOP on 13th October 2009.
- 28. Attended Project Review Committee meeting on Cellular and Molecular Biology of BMS Division, ICMR, New Delhi held on 27th October 2009.
- 29. Delivered a lecture on "Molecular Biology of Cancer by Genome Wide Approach" at CME in Pathology at Maulana Azad Medical College on 18th Nov. 2009.

- 30. Attended meeting on Research Data Repository & Business Intellegence (BI) held at ICMR, New Delhi on 8th December 2009.
- 31. Chaired Ethical Committee meeting of Safdarjang hospital, New Delhi on 15th December 2009.
- 32. Attended Project Review Committee meeting of NCD Division held at ICMR, New Delhi on 17th December 2009.
- 33. Conducted inspection of B.R. Singh Hospital & Centre for Medical Education & Research, Eastern Railway, Kolkata, on 18th December 2009 for renewal of accreditation for conducting DNB course of NBE, New Delhi.
- 34. Attended APCON-2009 organized by IAPM in Kolkata during $18^{th} 20^{th}$ December 2009.
- 35. Conducted interview for the selection of DNB Trainee for DNB Training Programme 2010 at Institute of Pathology on 4th January 2010.
- 36. Invited to present a talk on "Understanding molecular biology of cancer using genomic approach" in National Symposium on Current Trends in Genomics & Proteomics organized by Deptt. of Biotechnology, Deshbandhu Gupta college, University of Delhi, New Delhi on 4th -5th February 2010.
- 37. Attended ICMR-European Union (EU) Workshop in areas of cancer and neurosciences held at ICMR on 18th 19th February 2010.
- 38. Invited to give talk on "Esophageal cancer in north east India Contribution of genetic vis-à-vis environmental factors" held during 20th 23rd February 2010 at Amrita Institute of Medical Sciences and Research Centre, Cochin.
- 39. Attended 5th AOHUPO Congress held at Centre for Cellular and Molecular Biology (CCMB), Hyderabad as joint event with 14th DNAT convention and 1st Conference of the Proteomic Society of India (PSI) held on 24th February 2010.
- 40. Attended Technical Committee meeting held at ICMR on 26th February 2010.
- 41. Attended Screening Committee meeting for the Selection of Scientist 'E' held in ICMR on 26th February 2010.
- 42. Attended Technical Committee meeting held at ICMR on 16th March, 2010.
- 43. Invited to attend Selection Committee meeting for award of PDF of ICMR held at ICMR, New Delhi during 25th 27th March 2010.

Dr. Aruna Singh

- 1. Invited to attend symposium on "Commercialization of biotechnology" at India International Centre, New Delhi on 6th March 2009 organized by Biotechnology, Consortium of India Ltd (BCIL).
- 2. Invited to give key note address at "National conference on emerging trends in life sciences research" held at Birla Institute of Science and Technology, Pilani on $6^{\text{th}} 7^{\text{th}}$ March 2009.
- 3. Nominated to attend meeting on "Sustainable national vaccine policy" at NISTADS, CSIR from 4th 5th June 2009 to give a lecture on 'Need for new vaccine *–Chlamydia trachomatis'*.
- 4. Member Selection Committee for Integrated Program (M.Sc., Ph.D) at Dr. B. R. Ambedkar Center, University of Delhi on 7th June 2009.

Dr. Sujala Kapur

- 1. Attended 6th Asia Pacific Indian Association of Pathologists (IAP) Congress, 20th -23rd August 2009, Kochi.
- 2. Attended 58th Annual Conference of IAPM, Science City, Kolkata during 18th –23rd December 2009.
- 3. Invited to deliver a talk on "Tobacco and pesticide related cancers in north-east" India during National Institute of Advanced Studies (NIAS)-DST Programme on Gender Issues in Indian Science, Bangalore.
- 4. Invited to deliver a talk on "Immunogenetic profile of nasopharyngeal cancer". BBCI, Guwahati, 2009.
- 5. Attended PRC for review of projects on cancer, Division of NCD, ICMR Headquarters, New Delhi, 2009.
- 6. Attended PRC for review of projects on Gastroenterlogy, ICMR Headquarters, New Delhi, 2009.
- 7. Attended Brainstorming session on prevalence and prevention of NPC in north-east India. Organized by DBT and BBCI, Guwahati, 2009.
- 8. Attended Chapter and Annual meetings of IAPM, New Delhi.
- 9. Attended Conference of Organisation for Oncology and Translational Research (OOTR): 6th Annual Conference on 26th and 27th February 2010 at Kyoto, Japan.

- 10. Attended First NIAS-DST Programme on Gender Issues in Indian Science on Transcending Barriers to Performance: The Indian woman in Science. Bangalore, March 22 to 26, 2010.
- 11. Invited to deliver a talk on "DNA microarray in cancer: Principles, methodologies and implications" in Workshop on Molecular Biology Techniques in Cancer Diagnosis and Treatment at Rajiv Gandhi Cancer Research Institute and Research Centre, New Delhi in March 2010.

Dr. Sangita Rastogi

- 1. Deputed to participate in Training Workshop on '*Applications and importance of laboratory animals in biomedical research*' at NIRRH, Mumbai during 22nd-25th April 2009.
- 2. Assisted in compilation of Performance Budget of the Institute for onward submission to ICMR headquarters (2009).
- 3. Attended ICMR Prize Distribution ceremony for award to popular medical books in Hindi at ICMR headquarters, New Delhi on 5th May 2009.
- 4. Participated in seminar on '*Workflow solutions for identifying genetic variation*' organized by M/S Genetix Biotech Asia (P) Ltd. at Indian Habitat Centre, New Delhi on 3rd September 2009.
- 5. Attended Meeting on *'Advances in Rheumatology- Concepts to practice'* at AIIMS, New Delhi on 11th October 2009.
- 6. External examiner for Ph.D. (Zoology) thesis evaluation by CCSU (2009).
- 7. Participated in international symposium on '*Molecular pathology and applied genomics*' organized by Religare SRL Diagnostics at Indian Habitat Centre, New Delhi during 6th-7th November 2009.
- 8. Attended national symposium on *'Translational research in health sciences'* at AIIMS, New Delhi on 24th November 2009.
- 9. Examiner for M.Sc. (Toxicology) Part I examination, CCS University (2009).
- 10. Faculty for Ph.D. students registered with BITS, Pilani at Institute of Pathology, New Delhi.
- 11. Chaired Screening Committee Meeting for the post of Scientist 'B' held at Institute of Pathology (2009).

- 12. Attended series of lectures on '*Emerging technologies in life sciences*' during Research Solutions Seminar organized by ILS-Lonza at ITC Sheraton, New Delhi on 15th January 2010.
- 13. Invited talk titled, '*Chlamydia trachomatis infection in spontaneous aborters*' delivered at ICRH-2010 organized by ISSRF at Jaipur during 8th-10th February 2010.
- 14. Assisted in compilation of write-up of the Institute's research achievements for onward inclusion in Annual Report of DHR (2010).
- 15. Reviewer for international project proposal submitted to ICMR (2010).
- 16. Attended Selection Committee Meeting for the selection of Senior Research Fellow at Dept. of Microbiology, Safdarjung hospital, New Delhi (2010).

Dr. Poonam Salotra

- 1. Participated in the meeting of Molecular Immunology Forum held at Mumbai in March 2009.
- 2. Attended ICMR 4th Medical Development Congress on Stem Cell Research and Therapy at ASSOCHAM House, New Delhi on 13th 14th April 2009.
- 3. Participated in Symposium on "Self reliance through Innovation in Science & Technology" at NIPGR, New Delhi on 2nd May 2009.
- 4. Participated in the Steering Committee Meeting *of Leishmania* Vaccine project at Madrid, Spain in June 2009.
- 5. Participated in the Steering Committee Meeting of *Leishmania* Drug project at Antwerp, Belgium in October 2009.
- 6. Participated in workshop conducted by Voluntary Health Association of India, Program on Policy Issues in Kala-Azar in South-East Asia, Qutab Institutional Area, New Delhi on 27th November 2009.
- 7. Invited as a member of PRC meeting on Malaria, *Leishmania* and Filariasis held at ICMR, New Delhi on 8th December 2009.
- 8. Invited speaker at 97th Indian Science Congress held at Kerala University, Thiruvananthapuram in January 2010.
- 9. Appointed as a reviewer for several project proposals submitted to DBT, DST, ICMR etc.
- 10. Appointed as a reviewer for several manuscripts submitted to international journals.

Dr. Usha Agrawal

- 1. Deputed to attend the "Brainstorming Session" on "Data Repository and Management" in ICMR Hqs.
- 2. Served on the organizing committee of IAPM, Delhi Chapter Meet held at Institute of Pathology on 5th December 2009.
- 3. Attended IAPM Delhi Annual Chapter meet at Lady Hardinge Medical College, New Delhi, 2009.
- 4. Attended Guest Lecture given by Dr. S. Shriramachari to commemorate the 25th Anniversary of the Bhopal Gas Disaster given at Patel Chest Institute on 3rd December 2009.

Dr. LK Yerneni

1. Invited to deliver a talk entitled "Burn Injury: A Challenge For Tissue Engineers" at a Plenary Session on Regenerative Medicine held at Nichi-in Centre of Regenerative Medicine (NCRM), Chennai, on 24th October 2009.

Dr. Avninder Pal Singh

- 1. Presented an oral paper in 5th International Dermatopathology Conference at India Habitat Center from 6th 8th November 2009.
- 2. Participated in "Hands-on Training Course in Quantitative Proteomics" at Center for Cellular and Molecular Biology, Hyderabad during 26th Feb. 12th March 2010.

Dr. Ashwani K. Mishra

- 1. Attended "31st Annual Conference of the Indian Association for the Study of Population (IASP)" held at Tirupati, October 2009 and presented paper entitled "Regression approaches in understanding the role of vitamin D receptor gene polymorphism(s) in breast cancer risk in north Indian population".
- 2. Attended "27th Annual National Conference of Indian Society for Medical Statistics", held at Banaras Hindu University, Varanasi (UP), November 2009 and presented paper entitled "Vitamin D receptor gene polymorphism(s) and its association with breast cancer risk in north Indian population-A multinomial logistic regression approach".
- 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 Government of India, Ministry of Science of Technology, Government of India.

- 4. Involved as Co-Guide for the thesis dissertation work entitled "Role of epithelial mesenchymal transition in progression of bladder-Immunohistochemical analysis" as part of the DNB training programme at the Institute of Pathology, New Delhi.
- 5. Faculty for the course no. BIO-G532 'Biostatistics and Biomodelling' under the offdistance campus Ph.D programme in collaboration with BITS, Pilani for the session 2009-10.
- 6. Attended Project Review Meeting on project entitled "Comparative study on HIV/AIDS with ART with add-on homoeopathic drugs" approved by Centre Council for Research in Homoeopathic and Siddha, AYUSH, Ministry of Health and Family Welfare, New Delhi, India, February 2010.
- 7. Delivered lecture on 'Techniques of Data Integration" at the International Workshop on "Molecular and GIS based Epidemiology of Leprosy", at Institute of Pathology, New Delhi, March 2010.

DNB/ Ph.D PROGRAMME

DNB Programme

The Post-Graduate Level Training Programme in the speciality of Pathology continued during 2009-10. During the year, two students were admitted in the DNB course at Institute of Pathology:

1. Dr. Sherry Khanna

2. Dr. Manav Sawhney

The following two students who appeared for the DNB Theory Exam held in December 2009 have come out with flying colours:

- 1. Dr. Shweta Aggrwal
- 2. Dr. Binita Sinha
- 3. Dr. Sonal Agarwal

As per guidelines of the National Board of Examination, the Institute conducted Review Examinations of the DNB students in July 2009. Dr. Medha Tatke, Professor, Department of Pathology, GB Pant Hospital, New Delhi came as reviewer.

Ph.D Programme

- 1. **Mr. Bharat Bhushan** was conferred with the degree of Doctorate of Philosophy for his work on Acute Leukaemia by Jamia Hamdard in Dec. 2009.
- 2. **Mr. Rishein Gupta** was conferred with the degree of Doctorate of Philosophy for his work on Chlamydiasis by BITS-Pilani in Dec. 2009.
- 3. Mr. Indranil Chattopadhyay was conferred with the degree of Doctorate of Philosophy for his work on Esophageal Cancer by BITS-Pilani in 2009.
- 4. **Ms. Anuruppa Chakroborty** was conferred with the degree of Doctorate of Philosophy for her work on Breast Cancer by Guru Govind Singh Indraprastha University in 2009.
- 5. **Mr. Harsh Vardhan** was conferred with the degree of Doctorate of Philosophy for his work on Chlamydiasis by BITS-Pilani in 2010.

- 6. **Mr. Hem Chandra Jha** submitted his PhD thesis on Chlamydiassi to BITS-Pilani in Feb. 2010.
- 7. 2 students are currently registered for the Off-Campus Ph.D. programme of BITS-Pilani.

During 2008-09, Institute of Pathology further attracted young researchers- Research scholars with CSIR Junior Research Fellowships (2), ICMR Senior/Junior Research Fellowships (14) and UGC Fellowships (2) 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 activities of Ph.D./DNB students

Pradeep Singh Chauhan

- 1. Chauhan Pradeep Singh, Ihsan Rakshan, Mishra Ashwani Kumar, Bhushan Bharat, Kaushal Mishi, Yadav Dhirendra Singh, Devi Thoudam Regina, Soni Abha, Chattopadhyay Indranil, Saxena Sunita, Kapur Sujala. Poster presented on "Glutathione S-transferase and Microsomal Epoxide Hydrolase Gene Polymorphisms and Risk of Acute myeloid leukemia" in the first international conference "Hematologic Malignancies: Bridging the Gap 2010" Feb. 5-7, 2010 Singapore City, Singapore.
- 2. Attended the "National Workshop of CCamp Hands-on Basic Flow Cytometry Course" organized by National Centre for Biological Sciences from $17^{\text{th}} 21^{\text{st}}$ December 2009 at TIFR, Bangalore.
- Attended the National workshop on "Molecular Cytogenetics: Cancer Cytogenetics (solid tissue) by FISH" organized by Department of Reproductive Biology at AIIMS from 24th - 29th August 2009.

Thoudam Regina

 Attended International Workshop on Molecular and GIS Based Epidemiology of Leprosy from 4th - 9th March, 2010 at Institute of Pathology.

Mishi Kaushal

- Saxena Sunita, Kaushal Wasson Mishi, Chatterjee Indranil, Bhatnagar A, Chintamani, Bhatnagar D, Kapur Sujala. Genome-wide analysis of DNA copy number variations in Indian breast cancer patients using high-density SNP arrays. 20th Asia Specific Cancer Conference, Japan (Poster), 12th –14th November 2009,
- Saxena Sunita, Kaushal Mishi, Chatterjee Indranil, Bhatnagar A, Chintamani, Bhatnagar D, Kapur Sujala. Genome-wide analysis of genetic alterations in breast cancer patients from Northeast India using 10k SNP arrays. Organisation for Oncology and Translational Research, 26th -27th February 2010, Kyoto (Poster).

Pragya Srivastava

1. Attended 'International Symposium on Molecular Pathology and Applied Genomics' on 6th -7th Nov. 2009 at India Habitat Centre, New Delhi, India.

