Annual Report 2003

Institute of Cytology and Preventive Oncology,Noida

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INSTITUTE OF CYTOLOGY AND PREVENTIVE ONCOLOGY (ICMR)
MAULANA AZAD MEDICAL COLLEGE CAMPUS
NEW DELHI 110002

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PREFACE

The Institute continued its research activities primarily in cervical and breast cancer towards its control through primary and secondary approaches.

During these years, the Institute remained confined to its present location at Maulana Azad Medical College with inadequate space and infrastructure. Inspite of these inadequacies, it had contributed significantly towards research on cervical cancer. The contribution of the Institute spanned from clinical to basic. It had made contribution on the understanding of the molecular basis of cervical cancer development, specially the molecular genetic aspect and molecular mechanism of HPV infection. It also developed low cost feasible alternative strategy for early detection of cervical precancerous and cancerous.

As a follow up of brain-storming sessions held during the past two years, a pictorial manual and a calendar for visual inspection for early detection of precanceorus and cancerous lesions was prepared under a collaborative efforts with DST. These have been circulated widely among national and international experts / agencies involved in the cervical cancer control programme. The Institute has been identified as a multinational programme partner by IARC for evaluating low cost single vs double freeze cryotherapy for precancerous lesions of uterine cervix. WHO has also identified ICPO for a multinational collaborative programme on HPV vaccination for primary prevention of cancer of the uterine cervix. These programmes are in various stages of progress. Extramural funding has been increased significantly through competitive projects from various funding agencies.

During the year, 10th plan document was finalized. The vision document along with EFC document for the development of the Institute's new campus at NOIDA was finalized and submitted a long term planning for the Institute. New scientific and technical staff have been

recruited and the process is continuing to attract good scientific staff to undertake the new programmes proposed during the Xth plan.

The Institute remained busy in shifting its laboratories from MAMC Campus to NOIDA campus. As a result the scientific work suffered considerably. The Institute is now have two campuses for the present. A part of clinical set up would continue in MAMC Campus to carry out ongoing research collaboration with different hospitals.

A.B.Mitra
Officer-in-Charge

Highlights of the work done during April 2002 to

March 2003.

The Institute continued its research and human resource development activities in cancer of the uterine cervix as the priority area. Major efforts had gone towards the cancer cervix both for primary and secondary prevention. The Institute had recently taken up various programmes on breast cancer, the incidence of which is on the rise and in some metropolitan cities breast cancer is the leading cancer. Two multidisciplinary studies, one on cervical cancer and the other one on breast cancer are in progress.

The proposed collaborative programme with IRCH on breast cancer will enable the institute to streamline breast cancer study from inflow of patients, detection of early lesions and their management.

The multidisciplinary study on cervical cancer involving cytomorphological, HPV, genetical and molecular approaches with an emphasis to identify reliable biomarkers and/or risk factors for early detection of cervical cancer is in progress. Under the study 20,598 women were enrolled for cytological screening. The cervical lesions were found to be cancer (0.28%), HSIL (0.39%), LSIL (1.1%), ASCUS (4.4%), AGUS (0.38%) and the rest were either inflammatory or negative.

The Institute pioneered in developing and assessing alternative strategies for detection of early precacancerous and cancerous lesions. As a follow up of the recommendation of guidelines for early detection of cervical cancer, a pictorial manual for visual inspection was prepared and widely circulated among national and international experts. This manual and the calendar prepared on visual inspection are expected to go a long way in developing human resources for early detection of cancerous and precancerous lesions of cervix and could be

used by NCCP for training personels of different levels both professional and para medical. The manual and the calendar have been circulated to the gynaecologists at the national level.

The study on regulation of HPV gene expression continued. 65 tumour biopsies have been analysed for HPV typing and expression of c-fos, c-jun, and fra-1 at the RNA and protein level in the last year. The study revealed differential expression of different members of AP-1 family as well as change in the dimeriziation pattern of these AP-1 family members which might be playing a crucial role in the progression of the lesion. The study showed a gradual increase of c-fos expression and the binding activity as the severity of lesion increased. Presently investigation on the role of potent herbal antioxidative agent curcumin (Curcuma longa), which is very commonly used in Indian system of Medicine for its activities at molecular level was undertaken. Initial results have revealed a very interesting results where curcumin has shown a time-dependent as well as concentration-dependent down regulation of HPV-18 oncogene expression as well as binding activity of AP-1 in cervical carcinoma cell lines, HeLa. Curcumin in 100μM conentration can selectively suppress HPV expression by 2-3 hr of treatment while the AP-1 binding activity decreases gradually in a time-dependant fashion and completely disappear by 4-5 hours of incubation.

In another study HPV16 E6 350 variant- specific PCR analysis showed nine cases to be positive for HPV16 prototype E6- 350 T while eleven specimens were positive for HPV16 variant E6-350G, resulting in an amino acid change from leucine to valine at amino acid position 83 (L83V). Out of the nine moderately differentiated squamous cell carcinomas 4 specimens were prototype (E6 350T) whereas 4 samples were 350G variant. Out of the 11 well-differentiated squamous cell carcinomas, 6 were prototype (E6 350T) and 5 were E6 350G variants. One sample showed presence of both the variant 350G and 350T type, which was later confirmed by sequencing of the E6 region.

Studies have been initiated to investigate DNA methylation pattern and the expression level of methylase enzyme and differential gene expression in cervical and breast cancer. This study along with genetic instability in precancerous and cancerous lesions would increase the understanding of the molecular mechanism in the development of these cancers.

In an another study on cervical cancer, telomerase activity was assessed earlier in 150 samples comprising of cancerous and precancerous lesions and normal controls. All the tumours and majority of dysplastic lesions (40/62) were positive for telomerase activity which showed a positive correlation with hTERT expression. However, hTR expression was observed in all tumour and dysplastic lesions. This study further revealed that hTR and hTERT both are up regulated in presence of HPV infection. Analysis of TRF length points out that telomere attrition is a late event during cervical carcinogenesis since no telomere length reduction was observed in either dysplastic lesions or controls but in cancers. Hence it may not be suitable as a marker. In contrast, observation of telomerase activity in cervical preneoplatic lesions indicates that telomerase is activated long before the cells enter the 'crisis'.

Observation of over-expression of TRF1 protein expression pattern reveals in normal control tissues with longer telomeres and absence of expression in invasive cervical carcinomas, suggests that TRF1 protein negatively, regulates telomerase activity.

In the study on genetic instability and LOH in cervical cancer preliminary results revealed that MSI in general has frequent but comparatively more frequent in cervical precancerous lesions specially at BAT 25 locus. Data confirms that D2S123 and BAT25 are equally susceptible and are good markers for the assessment of microsatellite instability in both precancer as well invasive cancers. The genetic alterations including microsatellite instability and loss of heterozygosity can provide new insights into the molecular mechanism of

cervical carcinogenesis suggesting the possibility of candidate tumor suppressor genes at 5p and 3p that are playing important role in the development of cervical cancer. MSI alongwith HPV infection appears to be a potential marker for detecting the disease in its early stage.

During the year, the multidisciplinary study on breast cancer programme in collaboration with IRCH could not be initiated and as a result the recruitment of breast cancer patient is not satisfactory. Spade work has been done to have strong collaboration with IRCH. However, a cumulative number of 70 breast cancers were studied for BRCA-1 and 2 were studied for few exons commonly associated with BRCA mutation but no mutation was observed. The collaborative programme is still awaiting council's approval.

In another study on breast cancer, a total of 105 tumour biopsies from sporadic cases and 28 blood samples from familial breast cancer patients were collected from surgical OPD of Lok Nayak and Sucheta Kriplani Hospital, New Delhi. All patients belonged to the age group of 20-75 years and in stages I, II, III and IV. All exons of BRCA1 and specific exons 2, 9, 11, 11a, 18 and 20 of BRCA2 which are frequently mutated in BRCA2 were analyzed by PCR-SSCP and automated DNA sequencer. Out of 105 sporadic breast cancer were analysed, five (4.76%) mutations comprising two mutations (2%) in exon 2, one (1%) in exon 11 of BRCA1 and two mutations (2%) in exon 2 of BRCA2 were detected by PCR-SSCP assay. Other exons of BRCA1 and BRCA2 gene showed no mutation. Out of 28 familial breast cancer analysed so far only two (7%) mutation could be detected in exon 2 of BRCA1 gene and no mutation in BRCA2 gene.

The 271 breast cancers were analyzed immunologically for c-erbB-2 oncoprotein (HER-2/neu), epidermal growth factor receptor (EGF-R) and estrogen receptor (ER). Overall, the overexpression of both c-erbB-2 oncoprotein and EGF-R showed an inverse association with ER and a direct association with metastatic involvement of lymph node and high

histological grade. Interestingly, the frequency of c-erbB-2 and EGF-R overexpression was significantly higher among postmenopausal cases in comparison with premenopausal cases. Further, only in postmenopausal cases, c-erbB-2 oncoprotein and EGF-R as well as their concomitant expression revealed a statistically significant association with ER. In peripheral blood samples, the mean percentage of $CD4^+$ lymphocytes were found to be lower in breast cancer patients than in controls. Moreover, T-lymphocytes ($CD3^+$) showed a continued diminution along with the treatment.

The study of genetic polymorphisms of GST M1 and GST T1 on susceptibility was initiated for cervical cancer. A multiplex polymerase chain reaction method was used to detect the presence or absence of the GSTM1 and GSTT1 genes in genomic DNA isolated from cases with cervical cancer (n=142) and normal controls (n=96). The GSTM1 and GSTT1 null polymorphism were studied in different cancers and the controls. Homozygous GSTM1*0 and GSTT1*0 genotype is detected by PCR amplification of portion of these genes where homozygous for null genotype give no amplification product. GSTM1*0 genotype (GSTM1 null) was observed in 57% (81/142) of the cervical cancer cases in comparison to 34.4% (33/96) in controls. Increased risk for null type was noted in cervical cancer cases with an odd ratio of 2.5 (95% CI 1.4-4.5), which was found statistically significant (p=0.001). A total of 19.7% (28/142) of the cases presented homozygous GSTT1*0 as compared to 12.5% (12/96) in controls. The O.R was found to be 1.7(95% CI: 0.8-3.8), which was statistically not significant.

Cervical cancer case had marginally higher proportion (19%) of cases that were null for both GSTM1 and GSTT1 as compared to controls (11.4%). In esophageal cancer cases 53.65 % (22/41) case were GSTM1 null and 63.42% (26/41) GSTT1 null. This study is in progress. In CML cases 42.66% were GSTM1 null and same proportion were null for GSTT1. These are the preliminary results and detail work will continue.

During the year, major efforts have gone towards developmental work of the Institute complex and shifting to the new campus. The new research-cum-clinical complex has been partly constructed A master plan along with EFC has been prepared for the development of the Institute. Soon the Institute has been shifted to the new complex, and started functioning.

3. UTERINE CERVICAL CANCER

3.1 Multimodal screening tools for early detection of cervical cancer and precancerous lesions

Cytology: A total of 840 women underwent for cytology screening of which 449 (53.4%) had negative or inflammatory smears, 128 (15.2%) ASCUS/AGUS, 133 (15.8%) LSIL, 88 (10.4%) HSIL and 57 (6.7%) invasive cancers. All these women underwent simultaneous colposcopy and directed biopsy whenever indicated. A total of 156 biopsy proven CIN2 + lesions were detected.

VIA: A total of 867 women underwent VIA screening of which 259 (29.8%) were VIA positive. Of the 156 biopsy proven CINII + lesions, 137 showed VIA positivity. Sensitivity of VIA was 87.8 and specificity 82.8.

HPV screening

HPV testing was completed in 338 women using hybrid capture II kit. The negative predictive value of (NPV) was as high as 99%. Sensitivity and specificity of CIN lesions was also very high.

3.2 Cytomorphological analysis of cervical smears

Cytoscreening

Under this project a total of 20598 cervical smears were screened till March 2003. Of these 10453 (50.7%) were normal. 7078 (34.4% were benign cellular changes and 1367 (6.6%) were epithelial cell abnormalities. Of the epithelial abnormalities, 910 (4.4%) were ASCUS, 236 (1.1%) were LSIL: 80 (0.39%) were HSIL : 9(0.04%) were SIL – NOS: 74 (0.36%) were malignant 58 (0.28%) were AGUS and 1700 (8.3%) smears were found to be inadequate

Follow up of ASCUS/AGUS

The cases of ASCUS and AGUS (reactive groups) are under follow up. Preliminary analysis shows the most of these cases have turned out to be either normal

or inflammatory . The persistent ASCUS and ASCUS-favouring SIL cases on further follow up by colposcopy and biopsy revealed mostly LSIL lesions. Very few significant lesions were picked up on follow up of these cases. A good cytohisto correlation was achieved in high grade lesions.

3.3 Biological behaviour of Human papilloma virus infection of uterine cervix in Indian women:

Till date, 1239 women attending the gynae OPD of LNJP and SKH were screened cytologically. 75 were unsatisfactory for evaluation. The results of cytoscreening of satisfactory smears are as follows:

Within normal limits	711
Benign cellular changes	410
Epithelial cell abnormalities	43
ASCUS	29
AGUS	1
LSIL	6
HSIL	4
Malignant	3

HPV testing by PCR was performed in 580 cases, using L1 primer. A total of 119 (20.5%) HPV positive cases were detected. However, 69.7% of these were negative for high risk type HPVs.

The results for further testing by high risk probes (16 and 18) in various cytomorphological categories are as follows:

Cytodiagnosis	L1 positive	6+	11+	16+	18+	HPV +ve BUT 6, 11, 16, 18-ve
WNL/BCC (549)	105(19.1%)	5	24	1	-	75(71.4%)
ASCUS (20)	5(25%)	1	-	1	-	3(60%)
LSIL(6)	4(66.6%)	-	1	-	-	3(75%)
HSIL(2)	2(100%)	-	-	-	-	2(100%)
Malig (3)	3(100%)	-	-	3	-	-
Total 580	119(20.5%)	6	25	5	-	83(69.7%)

The HPV positive cases are being further analysed for other high risk viruses to build up the cohort for follow up study. HLA study has been initiated in collaboration with Institute of Immunohaematology, Mumbai. Mr. JK Sharma, SRO is undertaking the HLA studies.

3.4. Transcriptional Control of Human Papillomavirus gene expression by Antioxidants

The specific “high risk” HPV types 16 and 18 cause cervical cancer and the oncogenic potentials of these viruses have been attributed to their E6 and E7 open reading frames whose products can functionally interfere with the cell cycle control by interacting with p53 and Rb proteins. The expression of genes is generally regulated by the interplay of sequence-specific DNA binding proteins called transcription factors. The activator protein 1 (AP-1) which is formed either by a homodimer of jun proteins or a heterodimer of jun and fos proteins derived from host cells has been found to play a key role in controlling expression of HPV oncogenes. It has been also revealed that treatment of cervical carcinoma cells with some specific antioxidant can bring about changes in AP-1 transcription complex which leads to complete suppression of HPV expression. We have looked for certain potent antioxidative agents of harbal origin such as curcumin (Turmeric) for its role in cervical cancer cells HeLa. Studies have been

carried out to analyse composition and their functional importance in tissue biopsies from women with cervical precancer and cancerr.

a) Increased DNA binding activity and differential expression pattern of AP-1 transcription factor in cervical cancer tissues.

Seventy five cervical tumour biopsies have been obtained from cancer clinic of Lok Nayak Hospital and genomic DNA, RNA and total protein have been isolated for HPV typing and analysis of expression of c-fos, c-jun and fra-1 at the level of RNA and protein has been studied by northern blotting and immunoblotting using antibodies raised against AP-1 members.

We found differential expression and binding pattern as well as change in the dimerization pattern of AP-1 family members. AP-1 binding was not detected in control as well as premalignant lesions but, it showed a very high binding activity in high-grade cervical lesions. EMSA as well as immunoblotting experiments confirmed that it is the Jun-B and c-Fos heterodimer and not the c-jun and c-fos that forms the increased binding activity of AP-1. The results indicated a higher binding activity of c-fos within the AP-1 transcription complex in cancerous cells, while c-jun and fra-1 bound very poorly. Although fra-1 was found to express in moderate to high level in normal tissues, it was completely absent from the AP-1 complex in cancer cells. c-jun expression was found to be increased in cancer tissues but surprisingly, it does not participate in DNA binding. The results showed a good correlation of gradual increase in c-fos expression and its DNA binding activity as the severity of lesion increased. Immunoblotting experiments also showed similar pattern of c-fos expression. In contrast, the reverse is true for fra-1 which showed very low or negligible expression in cancerous cells but a high expression in normal cells.

B) Curcumin, the principal active component of Turmeric selectively suppresses HPV expression and abolish AP-1 binding activity.

It is a well known fact that the antioxidative drugs interferes with the redox status of eukaryotic cells. We have investigated the role of potent herbal antioxidative agent curcumin the main active component of turmeric (Curcuma longa), which is very commonly used in cooking curry and in Indian system of medicine for variety of activities. Investigation with cervical cancer cell HeLa indicate a most interesting results. Curcumin in 100μM conentration can selectively suppress HPV expression by 2-3 hr of treatment while the AP-1 binding activity decreases gradually in a time-dependant fashion and completely disappear by 4-5 hours of incubation.

3.5. Development of simple 'Paper Smear' method for rapid detection of HPV infection.

Since human papillomaviruses (HPVs) are major pathogens associated with the development of cancer of the uterine cervix, reliable diagnosis of HPV infection, particularly the 'high-risk' types (16/18), may facilitate early identification of 'high-risk' populations for developing cervical cancer and may augment the sensitivity and specificity of primary cervical cancer screening programmes by complementing the conventional Pap test. A simple "paper smear" method has been developed for dry collection, transport and storage of cervical smears/ scrapes at room temperature for subsequent detection of HPV DNA by a simple PCR assay. Several types of biological specimens such as imprint biopsies, blood and fine-needle aspirates including method. Cervical scrapes and other body fluids were smeared (within 0.5-1 cm diameter) and dried on to sterile small slides made of Whatman 3MM filter paper, and stored individually at room temperature or at 4°C. A small piece (2-3 mm) of the paper smear was cut out with a sterile surgical blade, boiled in an eppendorf tube and used directly for PCR amplification. The quality and quantity of DNA derived from paper smear and the results of

PCR amplifications for HPV type 16,BRCA1 and p53 genes were identical to those obtained from the same samples following standard collection. This method is simple, rapid and cost-effective, and can be effectively employed for large-scale population screening, especially for regions where the specimens are to be transported from distant places to the laboratory. This method is under US patent application.

3.6 Detection of HPV by multiplex PCR and RFLP

A low cost method was developed for the detection of human Papillomavirus types 6, 11, 16, 18 and 33 including co-infections from the cervical swabs of the females attending gynaeoclogical out patient departments and cancer clinics. The method detects the five most prevalent HPV types commonly associated with cervical abnormalities. The technique involves RFLP of the approximately 450bp amplicon, obtained after the amplification of L1 region of HPV genome by MY09/11 consensus primers. MY09/11 primers are used routinely for HPV detection covering a broad spectrum of HPV types as compared to general primers GP5+/GP6+. 90% of the cervical carcinoma have been shown to contain some high risk HPV types, HPV-16, 18 and 33 and few other are associated with CIN and cervical cancer, whereas HPV type 6 and 11 are associated with genital warts (condyloma accuminata and flat genital warts). Interaction between different HPV types (especially type 6 and 16) have been found to increase the oncogenic potential and promoting immortalization. Hence the detection of co-infection is equally important to understand the biological behaviour of HPVs. The method detects the above five HPV types by digesting the PCR product of MY09/11 primers with Rsa-1 and resolving on 8% non-denaturing polyacrylamide gel. The method has advantage over other conventional methods of HPV typing, as it saves the cost and time for second PCR by type specific primers. Most of the PCR-RFLP studies show either use of multiple restriction enzymes with two round of PCR. In the present method, single restriction enzyme was used for RFLP, which after electrophoresis and ethidium

bromide staining provides easily distinguishable bands. Further it was found to be more consistent than multiplex PCR. In conclusion, this method was found to be less combursive, low cost and user friendly for the detection of HPV DNA from cervical swabs, both at clinical and research level.

3.7 Genetic polymorphism of E6/ E7 gene of high risk HPV

The early genes of the HPV type 16 genome such as E2, E4, E5, E6, and E7 are critical in the pathogenesis of HPV – associated cancer, since they regulate viral replication and transcription as well as immortalization and transformation of infected cells. Any change in the sequences of these genes may lead to altered biological function of the proteins encoded by these genes, which in turn may influence the natural history of the infection. Therefore identification of HPV16 variants is important for the rational designing of newer diagnostic and therapeutic interventions in cervical cancer as well as for vaccine development strategies. We studied E6, E7and LCR region of HPV16 genome by 350G/T variant specific PCR and direct nucleotide sequencing in 20 cervical cancer biopsies positive for high risk HPV 16 only.

a) HPV 350 G/T polymorphism*:*

HPV16 E6 350 variant- specific PCR analysis showed nine cases to be positive for HPV16 prototype E6- 350 T while eleven specimens were positive for HPV16 variant E6-350G, resulting in an amino acid change from leucine to valine at amino acid position 83 (L83V). Out of the nine moderately differentiated squamous cell carcinomas 4 specimens were prototype (E6 350T) whereas 4 samples were 350G variant. Out of the 11 well-differentiated squamous cell carcinomas, 6 were prototype (E6 350T) and 5 were E6 350G variants. One sample showed presence of both the variant 350G and 350T type, which was later confirmed by sequencing of the E6 region.

b) HPV sequence analysis of the 3' part of the LCR, E6 and E7:

Apart from 350G and T variant analysis of detailed sequence variation was performed by direct sequencing of the E6, E7 and LCR regions of HPV16 in eight samples. Direct sequencing of 3' region of E6 showed HPV16 prototype E6-350 T in four samples and four specimens were positive for HPV16 variant E6-350G, resulting in an amino acid change from leucine to valine at aminoacid position 83 (L83V). Apart from this variation no other variation was observed.

Sequencing of 3' region of E7 showed a variation from G to A at nucleotide position 666 in one sample and it was a silent mutation as it coded for the same amino acid leucine in one sample and all the other samples were similar to the prototype. HPV16 multivariants were observed in the LCR region. Sequencing of LCR region showed two type of variations: G to A at nucleotide position 7518, observed in seven samples and T to G at nucleotide position 7711, observed in two samples. Analysis on more number of samples is in progress.

3.8 Analysis of Methylation pattern in cervical cancer tissue samples

Recent developments in genetics of cancer, one could understand cancer causes not only through the conventional mechanisms of carcinogenesis, rather it includes new concept of regulation called epigenetics. One of the epigenetic mechanism includes “Methylation (of cytosines in genome)”.

Genome wide hypomethylation and regional hypermethylation with respect to tumor suppressor genes were found in cancers, which modifies the Knudson’s +Hypothesis of mutation in one allele and deletion in other allele, modification to be in addition to the above, Methylation in one allele, and mutation, or deletion or Methylation in both alleles were found.

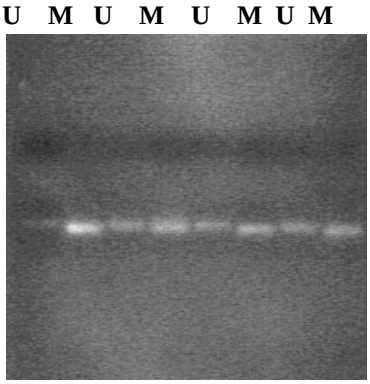
From the previous studies from our lab, it was found that chromosome region 3p was altered through the mechanism either through LOH, or mutation, and it is suspected whether Methylation could contribute in tumorigenesis process, we selected the genes that are harbored in 3p region as well other tumour suppressor genes.

Methylation pattern in selected genes RASSF1A, FHIT, MGMT,GSTP1 were taken in to consideration for the above mentioned study.

We have done few samples with RASSf1A, and fHIT, final confirmation of Methylation pattern has to be done with the help of sequencing.

RASSF1A Methylation PCR

Unmethylated and methylated PCR loaded in adjacent wells showing single allele methylated,and other unmethylated,which has to be confirmed with sequencing.



3.9. Role of Telomerase activity and its subunits hTR and hTERT during cervical carcinogenesis

Although infection of HPV is essential, the virus alone seems to be incapable of causing carcinogenic transformation of cervical epithelium. Involvement of certain cellular factors acting through specific molecular pathways seems to play a key role in HPV- induced cervical carcinogenesis. One such pathway we looked for is the activation of telomerase activity.

We observed a highly elevated level of telomerase activity in all invasive cancer and its gradual increase with the increasing severity of cervical lesions but its complete absence in normal controls suggests that telomerase activation is an important early event during the process of cervical carcinogenesis. Thus the level of telomerase activity may serve as a potential marker for cervical cancer screening programmes and prognosis of the disease. It is well established that high-risk HPV positive cervical lesions show a higher rate of progression. Also, observation of a higher level of telomerase activity in cytologically diagnosed normal cases such as inflammation and ASCUS positive for high risk HPVs indicates that high risk HPV infection can activate telomerase expression. Thus detection of high risk HPVs coupled with telomerase assay would allow early identification of high risk population who are likely to progress to cervical cancer.

We have analysed telomerase components hTR, hTP1 and hTERT, which revealed that hTERT is the rate-limiting factor for telomerase activity and it positively regulates telomerase activity. Though hTR and hTP1 are essential components of telomerase but since they found to be expressed even in normal control cervical tissues, they do not seem to have any regulatory role and cannot serve as a diagnostic endpoint.

Analysis of TRF length points out that telomere attrition is a late event during cervical carcinogenesis since no telomere length reduction was observed in either

dysplastic lesions or controls but in cancers. Hence it may not be suitable as a marker. In contrast, observation of telomerase avtivity in cervical preneoplatic lesions indicates that telomerase is activated long before the cells enter the 'crisis'.

Observation of over-expression of TRF1 protein expression pattern reveals in normal control tissues with longer telomeres and absence of expression in invasive cervical carcinomas, suggests that TRF1 protein negatively, regulates telomerase activity.

3.10. Phase III Clinical trial of Polyherbal Neem Cream and Tablet-'Praneem' in women with HPV infection.

Praneem polyherbal cream and a polyherbal tablet which have inhibitory action on a wide spectrum of genital tract pathogens such as Herpes simplex-2, Chlamydia trachomatis have been developed for intravaginal use. It has been observed that women infected with high-risk HPV types HPV 16/18 show a high rate of progression to cervical cancer. Besides HPV, involvement of other sexually transmitted agents such as Chlamydia trachomatis, N. gonorrhea, HSV-2 has been suggested as co-factors for cervical carcinogenesis. It would be interesting to see the effects of this herbal antimicrobial cream/ tablet in women with HPV infection. A study has therefore been planned to see the efficacy of this cream/tablet in women with HPV infection.

Clinical trials are in progress, at Lok Nayak Hospital, New Delhi. In out patients Department, women attending gynaecology and obstetrics with complaint of genital infections causing abnormal discharge were recruited for the purpose of the study. About 150 women have been screened for the presence of HPV sequences by PCR and six women with HPV16 positivity were give the Praveen tablet for 4 weeks. Out of 4 patients analysed 2 showed absence of HPV while one patient felt relieved. Trials is in progress in more cases.

3.11 Loss of heterozygosity

Analysis of loss of heterozygosity was done using five different polymorpohic markers for the chromosomal arms 3p (3p14.1-14.2) and 5p(5p15.1-15.2) for their reported high LOH rate.

Primers (LOH analysis)

Markers	Amplimer	Amplicon size (bp)
D3S1234	5'-CCT GTG AGA CAA AGC AAG AC-3' 5'-GAC ATT AGG CAC AGG GCT AA-3'	111
D3S1300	5'-AGC TCA CAT TCT AGT CAG CCT-3' 5'-GCC AAT TCC CCA GAT G	236
D3S1313	5'-CCC CTT GGA AAA TCA CTG-3' 5'-CCA TGA ATA AGC CTT GCC-3'	233
D5S208	5'-ACC TGA GTC TTC ATC AAT AC -3' 5'-TCC AGA ATC ATC CAT GTT GT-3'	186
D5S406	5'-CCT GCC AAT ACT TCA AGA AA-3' 5'-GGG ATG CTA ACT GCT GAC TA-3'	185

Incidence of LOH in precancer was significantly low (<25%) as compared to invasive cancer, maximum LOH was observed on chromosome 5p (D5S208 & D5S406).Highest LOH score in precancers was 13.3% at 5p (D5S406) and 10.7% at 3p14.2 (D3S1300). No significant correlation was found between HPV infection and LOH in precancers, however HPV positivity in cases exhibiting LOH in invasive cancers was significantly higher.

Human papillomavirus infection

To see the prevalence of HPV infection among different grades of lesions 473 females including those with normal cervix, chronic cervicitis, mild, moderate, severe dysplasia, squamous cell carcinoma and adenocarcinoma were analyzed using MY09/11 consensus primers and type specific primers for four most prevalent HPV types (6, 11, 16, and 33). Out of 473 cases 178, (37.6%) were found HPV positive by

consensus primers (MY09/11), HPV was present in normal cervix (24.7%), chronic cervicitis (37.5%), mild dysplasia (36.0%), moderate & severe dysplasia (57.5%), squamous cell carcinoma (85.2%), adenocarcinoma (100%). Of the above 473 cases examined, maximum infection in all categories was found in the ages between 30 and 50 years. . Infection with HR-HPV types (especially type 16) was in about 7% in severe dysplasia, no case of HPV type 16 was found in women with normal cervix in chronic cervicitis HPV 16 was 3.1%, However, type 16 was significantly higher in invasive cancer (42% in SCC and 33% in adenocarcinoma) HPV type 18 was present in 66% cases of adenocarcinoma. However, the incidence of low risk-HPV type (especially type 6) was quite high in both dysplasia (18.2% in mild dysplasia and 22.2% in severe dysplasia) and invasive cancers (75% in SCC and 33.3% in AC). Co-infections of type 6 and 16 were significantly high in SCC (26.2%).

Human papillomavirus infection and MSI in precancer

A significant correlation was found between the HPV infection and microsatellite instability in precancerous lesions of uterine cervix. In precancer HPV positivity was 48.2% (36% in mild dysplasia and 57.5% in severe dysplasia. In both, mild dysplasia and severe dysplasia HPV positivity was significantly higher ($p=<0.01$) with MSI (64.5%) as compared to MSS (29.6%). HPV positivity in severe dysplasia with MSI-L was 58.3% as compared MSI-H (85.7%). Overall HPV positivity in severe dysplasia was high as compared to HPV in mild dysplasia though the difference was not significant.

3.12 Genetic instability and LOH in cervical cancer

Analysis of loss of heterozygosity and microsatellite instability was done on 58 dysplasia (25 mild to moderate and 33 severe dysplasia) and 100 squamous cell carcinoma (moderately to well differentiated) of uterine cervix.

Microsatellite instability

Microsatellite instability was tested using the Bethesda Consensus Conference reference panel of five markers, Bat-25, BAT-26, D2S123, D5S346 and D17S250 (Boland, et al. 1998).

Primer details

(MSI analysis)

Primers	Chromosomal arms	Repeat motifs	Amplicon size (bp)
BAT 25	4q12	(T).A(T)25	~90
BAT 26 2p	(T)5.	(A)26	~80-100
D5S346	5q21/22	(CA)26	~96-122
D2S123	2p16	(CA)13.TA(CA)15. (T/GA)7	~197-227
D17S250	17q11.2	(TA)7…(CA)24	~150

Cases exhibiting MSI with one primer were considered as MSI-L, whereas those with instability on two or more loci were considered to be MSI-H. Cases that do not show instability with any of the five primers were considered as microsatellite stable (MSS).

Microsatellite instability was found to be an early event in the process of cervical carcinogenesis. 31 out of 58 (53.4%) precancer cases studied, showed instability. [MSI-H =15.5% and MSI-L = 37.9%]. Of which 48% (12/25) of the cases with mild dysplasia showed instability [MSI-H = 8.0% and MSI-L = 40%]. In contrast to early cervical lesion an increased frequency of microsatellite instability was observed in severe dysplasia showing 57.5% (19/33) MSI [MSI-H = 21.2% and MSI-L = 36.3%]. Whereas 52% of the mild dysplasia and 42.4% of the severe dysplasia cases were microsatellite stable (MSS), which appear to characterize two different pathways of

carcinogenesis. In precancers maximum instability (27.5%) was found in the 2p16 region (D2S123) where the repeat motifs were (CA)13 TA (CA)15 (T/GA)11, whereas BAT25 also showed a significantly higher instability (20.6%), BAT 26 showed little instability in the precancerous lesions. Maximum instability (22.5%) was observed at the intron 5 of c-kit oncogene (BAT-25) in invasive cancers where, in addition to MSI, LOH 3.57% was also observed. BAT-26 showed little instability in invasive cancers (10%) but high frequency of LOH (26.31%) was observed with the same marker. Study indicated that certain loci are more susceptible to instability. we, in our study took five internationally recommended primers for mono and dinucleotide repeats for 58 precancer including 25 mild and 33 severe dysplasia and 100 invasive cervical cancer. Our data confirms that D2S123 and BAT25 are equally susceptible and are good markers for the assessment of microsatellite instability in both precancer as well invasive cancers. The genetic alterations including microsatellite instability and loss of heterozygosity can provide new insights into the molecular mechanism of cervical carcinogenesis suggesting the possibility of candidate tumor suppressor genes at 5p and 3p that are playing imoprtant role in the development of cervical cancer. MSI alongwith HPV infection appears to be a potential marker for detecting the disease in its early stage.

3.13 Role of GST polymorphism in various cancers.

The glutathione S-transferase supergene family is an important part of cellular enzymic defense against endogenous and exogenous chemicals, many of which have a carcinogenic potential. However, while a wide variety of chemicals can act as substrates for different members of the supergene family, the precise function of these enzymes remain unclear. The supergene family comprises several gene families that include polymorphic loci, prompting the hypothesis that allelic variants associated with less effective detoxification of potential carcinogens can confer an increased susceptibility to cancer. For example, the null genotypes at the mu class GSTM1 and theta class GSTT1

loci have attracted particular interest, and recently identified allelic variants at the mu class GSTM3 and pi class GSTP1 loci are also putative susceptibility candidates. Associations between GSTM1 and GSTT1 genotypes and risk have been observed in some case-control studies in lung, bladder and colon cancers. Influence of glutathione S-transferase polymorphisms on the risk of several cancers, including basal cell carcinoma of skin has been observed, suggesting a role for GST enzymes in the detoxification of the products of ultraviolet radiation-induced oxidative stress.

The glutathione S-transferases (GSTs) comprise a supergene family of phase 2 detoxifying enzymes that catalyse a variety of reduced glutathione-dependent reactions with compounds containing an electrophilic center. GST enzymes appear to be expressed in most, if not all, life forms, a finding that suggests their importance in the protection of cells from harmful chemicals. In humans, the enzymes expressed in tissue cytosols have been most intensively studied, and four major gene families have been identified: alpha on chromosome 6, mu on chromosome 1, theta on chromosome 22, and pi on chromosome 11. Sequence data on enzymes of the different classes suggests the ancestral cytosolic GST gene was of the theta class with progressive divergence of the sigma class and then of the mu class GST. A membrane-associated GST that evolved separately to the cytosolic enzymes has, been identified, although its influence on cancer susceptibility is unknown.

a) Polymorphism in glutathione S-transferase genes

There is evidence for allelism in GST genes in each of the alpha, mu, theta and pi gene families. Alpha class gene family consists of two or three functional genes and at least four pseudogenes on chromosome 6p12, which appear to have evolved by gene duplication and gene conversion events. GSTA1 and GSTA2 are the two genes in this family that are well characterized and one restriction fragment length polymorphism is reported in GSTA2. Five mu genes (GSTM1-GSTM5) situated in tandem on

chromosome 1p13 have been identified. Three alleles has been reported at GSTM1 locus namely GSTM1*0, GSTM1*A and GSTM1*B. GSTM1*0 (null) is deleted and individuals homozygous for this allele express no GSTM1 protein. GSTM1*A and GSTM1*B differ by only a single base in exon 7 and encode enzyme monomers that form active homo and heterodimeric enzymes. GSTM3 also show polymorphism and some individual show no M3 enzyme activity suggesting gene deletion like GSTM1. Two alleles of GSTM3 has been identified GSTM3*A and GSTM3*B (3 base pair deletion in intron 6). Theta class gene family has two genes GSTT1 and GSTT2. GSTT1 shows null polymorphism (GSTT1*0). Pi class gene family has only one functional gene and show polymorphism. We have reported one BamHI RFLP polymorphism in Indian population. Other reported polymorphisms are polymorphism in the region of pentanucleotide repeats (ATAAA) in the 5' promoter region with unclear phenotype. There are other two alleles (SNP's) GSTP1*B and GSTP1*C wild type being GSTP1*A.

It is reasonable to speculate that homozygosity of null allele or those encoding for low activity variants are associated with biochemical consequence. Keeping above fact in mind we have carried out some case control studies to look for the role of these polymorphisms in cancer susceptibility.

b) GST polymorphism in cervical cancer, esophageal cancer, and CML

We have looked for the GSTM1 and GSTT1 null polymorphism in different cancers and the controls. Homozygous GSTM1*0 and GSTT1*0 genotype is detected by PCR amplification of portion of these genes where homozygous for null genotype give no amplification product. GSTM1*0 genotype (GSTM1 null) was observed in 57% (81/142) of the cervical cancer cases in comparison to 34.4% (33/96) in controls. Increased risk for null type was noted in cervical cancer cases with an odd ratio of 2.5 (95% CI 1.4-4.5), which was found statistically significant (p=0.001).

A total of 19.7% (28/142) of the cases presented homozygous GSTT1*0 as compared to 12.5% (12/96) in controls. The O.R was found to be 1.7(95% CI: 0.8-3.8), which was statistically not significant.

Cervical cancer case had marginally higher proportion (19%) of cases that were null for both GSTM1 and GSTT1 as compared to controls (11.4%).

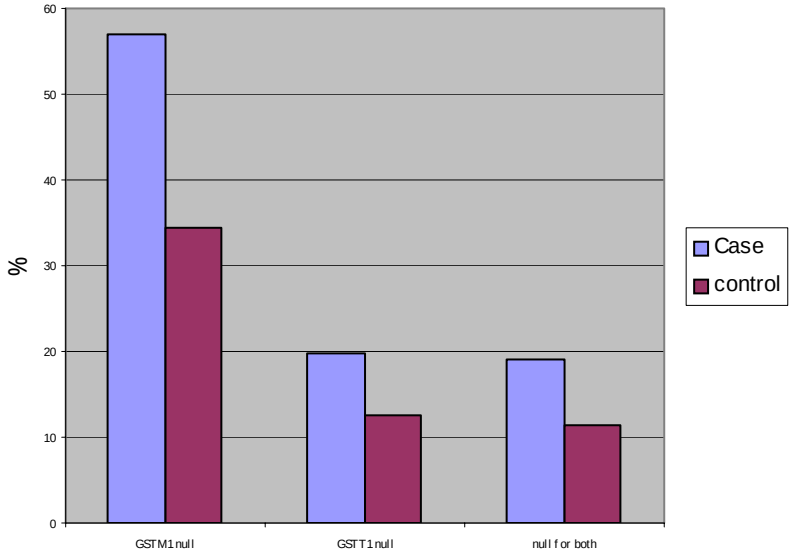
In esophageal cancer cases 53.65 % (22/41) case were GSTM1 null and 63.42% (26/41) GSTT1 null. This study is in progress.

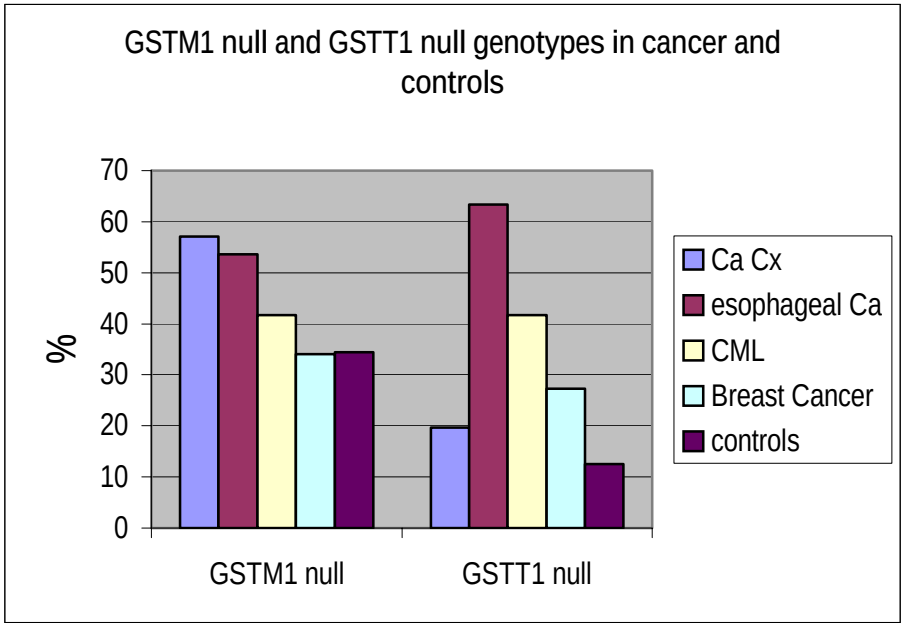
In CML cases 42.66% were GSTM1 null and same proportion were null for GSTT1. These are the preliminary results and detail work will continue.

GST studies in COPD (chronic obstructive pulmonary disease) cases

The role of GST polymorphisms in COPD has been studied in 50 COPD cases and equal number of age and smoking habit matched controls. Null polymorphisms of GSTM1 and GSTT1 are being analyzed for these cases. The work is in progress. Since environmental pollutants it is expected cause COPD that polymorphisms of detoxifying enzyme may play an important role.

GSTM1 and GSTT1 null proportion in cervical cancer cases and controls





4. BREAST CANCER

4.1 Multidisciplinary study

Breast cancer is the commonest cancer in women worldwide. In India also it is on the rise specially in the metropolitan cities as is reflected in the NCRP report (1992-1996). It is number one cancer in Mumbai (Annual Incidence Rate 29) while in Delhi also it is number one cancer now (Annual incidence rate 28). Incidence rates begins to rise in the early thirtees and reaches a peak in the 50-65 age group.

The information on the epidemiology of breast cancer in India and also the genetic aspect is very limited except a few report in limited sample.

In view of the rising incidence of breast cancer amongst Indian women this project has been initiated with the following objectives

Specific objectives

a) Study of epidemiological risk factors related to breast carcinogenesis

b) Identification of various clinical and morphological prognostic parameters

c) Estimation of plasma levels of androgens and their binding globulins in breast cancer cases

d) Analysis of immunoexpression of estrogen regulated genes in breast cancer.

e) Molecular studies on detection and regulation of breast cancer genes (BRCA1 & 2) and suppressor genes including p53.

f) Analysis of chromosomal instability, and c-erb B2 gene in breast cancer.

Results on 100 breast cancer cases was reported in the last annual report.

The recruitment of all the subjects under study was done based on cytological diagnosis by fine needle aspiration. The biological material for breast cancer susceptibility

gene studies as well as for various prognostic factors analysis has been collected following the cytological diagnosis. The epidemiological data pertaining to study the high risk factors has also been noted down for all the study subjects on a predesigned proforma.

To improve upon this aspect (poor recruitment) as well as to study the role of various prognostic factors on patient survival (both overall as well as disease-free) a collaboration with IRCH, AIIMS has been finalized during the last year. Since the desired financial support could not be provided to IRCH during the year under report, the institute could not utilize this clinical base for the purpose of this project. However, the efforts are being made to sort out this and hope to improve the enrolment of the patient as desired in the project. Based on Council's comments, the project had been referred to IRCH for re-ethical clearance.

4.2 Immunohistochemical expression of c-erbB-2 oncoprotein (HER-2/nu) and epidermal growth factor receptor (EGF-R) in pre- and postmenopausal breast cancer.

Several lines of research from epidemiological, clinical and experimental angles indicate that estrogens play an important role in the etiology of breast cancer. Interestingly, breast cancer progression is related to a gradual loss of estrogen requirement for tumor growth. The escape from hormonal control signifies the poor prognosis, and may be associated with c-erbB-2 overexpression. The c-erbB-2 gene is located on chromosome 17 and encodes a 185 kilodalton transmembrane receptor-like phosoglycoprotein that is closely related in structure but is still biologically distinct from the EGF-R. The c-erb B-2 oncoprotein has been frequently cited as a marker of poor prognosis in breast cancer. The objective of the study was to evaluate the overexpression of EGF-R and c-erbB-2 oncoprotein in breast cancer and their relation with different prognostic parameters such as grade of the tumors, nodal and receptor status. Breast cancer tissues from271 cases were analysed immunohistochemically for c-erb B-2 oncoprotein, EGF-R and estrogen receptor (ER). Overall, the overexpression

of both c-erbB-2 oncoprotein and EGF-R showed an inverse association with ER and a direct association with metastatic involvement of lymph node and high histological grade. Interestingly, the frequency of c-erbB-2 and EGF-R overexpression was significantly higher among postmenopausal cases in comparison with premenopausal cases. Further, only in postmenopausal cases, c-erbB-2 oncoprotein and EGF-R as well as their concomitant expression revealed a statistically significant association with ER. The investigated pathological factors of the present study indicate a difference between pre- and post menopausal breast cancer.

Table 1. Overexpression of c-erbB-2 oncoprotein and EGF-R, and their relation with different pathobiological factors in breast cancer.

		c-erbB-2		EGF-R		Concomitant c-erbB-2 & EGF-R overexpression			ER	
		Positive (n=114)	Negative (n=157)	Positive (n=112)	Negative (n=159)	Positive (n=65)	Both negative (n=110)	Positive to one (n=96)	Positive (n=93)	Negative (n=178)
Menopausal	Pre- (n=136)	48	88	46	90	26	68	42	46	90
	Post- (n=135)	66	69	66	69	39	42	54	47	88
		X^2=5.1, p<0.05*		X^2=6.3, p<0.05*		X^2=10.2, p<0.01*			X^2=0.03, p=0.9	
Grade	Low (n=80)	24	56	24	56	12	44	24	32	48
	Intermediate (n=129)	47	82	47	82	22	57	50	44	85
	High (n=62)	43	19	41	21	31	9	22	17	45
		X^2=25.4, p<0.01*		X^2=21.2, p<0.01*		X^2=38.7, p<0.01*			X^2=2.5, p=0.3	
Lymph node	Positive (n=171)	80	91	81	90	51	61	59	50	121
	Negative (n=100)	34	66	31	69	14	49	37	43	57
		X^2=4.2, p<0.05*		X^2=7, p<0.01*		X^2=9.5, p<0.01*			X^2=5.3, p<0.05*	
ER	Positive (n=93)	29	64	28	65	14	50	29	-	-
	Negative (n=178)	85	93	84	94	51	60	67	-	-
		X^2=6.9, p<0.01*		X^2=7.4, p<0.01*		X^2=11.5, p<0.01*				

*Significant

Table 2. Overexpression of c-erbB-2 oncoprotein and EGF-R, and their relation with various prognostic factors in pre- and postmenopausal cases of breast cancer.

Premenopausal (n=136)		c-erbB-2		EGF-R		Concomitant c-erbB-2 & EGF-R overexpression		
		Positive (n=48)	Negative (n=88)	Positive (n=46)	Negative (n=90)	Positive (n=26)	Both negative (n=68)	Positive to one (n=42)
Grade	Low (n=50)	10	40	13	37	5	32	13
	Intermediate (n=63)	23	40	20	43	11	31	21
	High (n=23)	15	8	13	10	10	5	8
		X^2=14.2, p<0.01*		X^2=6.8, p<0.05*		X^2=15.7, p<0.01*		
Lymph node	Positive (n=79)	33	46	31	48	19	34	26
	Negative (n=57)	15	42	15	42	7	34	16
		X^2=3.5, p=0.06		X^2=2.5, p=0.12		X^2=4.5, p=0.11		
ER	Positive (n=46	13	33	12	34	6	27	13
	Negative (n=90)	35	55	34	56	20	41	29
		X^2=1.5, p=0.22		X^2=1.9, p=0.17		X^2=2.6, p=0.28		
Postmenopausal (n=135)		c-erbB-2		EGF-R		Concomitant c-erbB-2 & EGF-R overexpression		
		Positive (n=66)	Negative (n=69)	Positive (n=66)	Negative (n=69)	Positive (n=39)	Both negative (n=42)	Positive to one (n=54)
Grade	Low (n=30)	14	16	11	19	7	12	11
	Intermediate (n=66)	24	42	27	39	11	26	29
	High (n=39)	28	11	28	11	21	4	14
		X^2=12.4, p<0.01*		X^2=11.7, p<0.01*		X^2=20.3, p<0.01*		
Lymph node	Positive (n=92)	47	45	50	42	32	27	33
	Negative (n=43)	19	24	16	27	7	15	21
		X^2=0.6, p=0.46		X^2=3.4, p=0.06		X^2=5, p=0.08		
ER	Positive (n=47)	16	31	16	31	8	23	16
	Negative (n=88)	50	38	50	38	31	19	38
		X^2=6.4, p<0.05*		X^2=6.4, p<0.05*		X^2=11.5, p<0.01*		

*Significant

T- lymphocytes in breast cancer: association with prognostic factors and response to treatment.

In patients with malignancies, including breast cancer, immunological status especially cell mediated immunity plays an important role in cancer development and prognosis. The immune system is capable of responding to breast cancer as evidenced by systemic, regional and intra tumoral lymphocytic activation. Nevertheless, lymphocyte infiltration in breast cancer can give information on both good and poor prognosis.

In the present study, T-lymphocyte subsets $CD4^+$ (helper) and $CD8^+$ (suppressor) cells were evaluated in breast cancer tissues as well as in the peripheral blood of breast cancer patients. Tissue sections were analysed immunocytochemically for $CD4^+$, $CD8^+$, interleukin-2 receptor (IL-2R), estrogen receptor (ER) and c-erbB-2 (HER-2/neu) oncoprotein overexpression. Majority of tumor tissues showed a preponderance of $CD8^+$ lymphocytes. Further, in majority of tumors, less than 25% of the tumor-associated lymphocytes were positive for IL-2R expression. Excepting an association between involvement of lymph node and IL-2R expression, lymphocyte subsets and IL-2R did not reveal any significant association with prognostic factors such as tumor grade, lymph node metastasis (for subsets), ER and c-erbB-2 positivity. On the other hand, in peripheral blood samples, the mean percentage of $CD4^+$ lymphocytes was found to be lower in breast cancer patients compared to controls. Moreover, T- lymphocytes ($CD3^+$) showed a continual diminution along with the treatment

Table 1, Shows the tumors with predominant $CD8^+/CD4^+$ lymphocytes along with IL-2R expression by infiltrating lymphocytes, and their association with prognostic factors:

		Mainly $CD8^+$ cells (n=13)	Mainly $CD4^+$ cells (n=3)	Equal $CD4^+$ & $CD8^+$ (n=9)	IL-2R expression		
					Up to 25% (n=15)	25-50% (n=8)	>50% (n=2)
Grade	Low (n=7)	5	0	2	2	4	1
	Intermediate (n=13)	5	2	6	9	3	1
	High (n=5)	3	1	1	4	1	0
				(p>0.05)			(p>0.05)
Lymph node	Positive (n=16)	10	2	4	7	8	1
	Negative (n=9)	3	1	5	8	0	1
				(p>0.05)			(p>0.04)
ER	Positive (n=9)	5	1	3	6	3	0
	Negative (n=16)	8	2	6	9	5	2
				(p>0.05)			(p>0.05)
c-erbB-2	Positive (n=11)	4	2	5	5	4	2
	Negative (n=14)	9	1	4	10	4	0
				(p>0.05)			(p>0.05)

* significant

Table 2, Mean, S. D. and statistical significance of T-cell population and subsets of peripheral blood in control women and breast cancer cases before and after treatment

Groups	$CD3^+$ (%)	$CD4^+$ (%)	$CD8^+$ (%)
Before treatment (n=42)	73.5±5.93	31.6±8.09	34.5±9.03
Post-radiotherapy(n=32)	69.9±6.09	32.5±8.25	37.6±7.35
Post-chemotherapy(n=30)	61.7±12.43	31.2±9.69	35.9±10.08
Normal controls (n=37)	76.4±10.28	49.4±17.33	33.2±11.87
Before treatment vs. post-radiotherapy	p<0.01*	p>0.05	p>0.05
Post-radiotherapy vs. post-chemotherapy	p<0.01*	p>0.05	p>0.05
Before treatment vs. post-chemotherapy	p<0.01*	p>0.05	p>0.05
Before treatment vs. controls	p>0.05	p<0.001*	p>0.05

4.3. BRCA1 and BRCA2 mutation in sporadic and familial breast cancer patients.

Breast cancer susceptibility genes BRCA1 and BRCA2 is responsible for majority of familial early-onset breast and ovarian cancer. The BRCA1 gene is located on to chromosome 17q12-21 and contains 22 coding exons, express mRNA of 7.8 kb with a nuclear protein product of 220 kDa. The BRCA1 gene responsible for 50% of inherited breast cancer and more than 80% of inherited breast and ovarian cancer. The BRCA2 gene with 26 coding exons located on chromosome 13q12-13 has been shown to predispose mainly to male breast cancer but it can also be seen mutated in familial breast cancer in females. Approximately, 5-10% of the breast cancer are believed to be hereditary in nature.

In view of limited information in the area of molecular biology of breast cancer in India, in the present study mutation of BRCA1, BRCA2 and p53 genes has been studied along with normal controls.

A total of 105 tumour biopsies from sporadic cases and 28 blood samples from familial breast cancer patients were collected from surgical OPD of Lok Nayak and Sucheta Kriplani Hospital, New Delhi. All patients belonged to the age group of 20-75 years and in stages I, II, III and IV. All exons of BRCA1 and specific exons 2, 9, 11, 11a, 18 and 20 of BRCA2 which are frequently mutated in BRCA2 were analyzed by PCR-SSCP and automated DNA sequencer. Out of 105 sporadic breast cancer were analysed, five (4.76%) mutations comprising two mutations (2%) in exon 2, one (1%) in exon 11 of BRCA1 and two mutations (2%) in exon 2 of BRCA2 were detected by PCR-SSCP assay. Other exons of BRCA1 and BRCA2 gene showed no mutation. Out of 28 familial breast cancer analysed so far only two (7%) mutation could be detected in exon 2 of BRCA1 gene and no mutation in BRCA2 gene.

4.4. p53 gene mutation in sporadic and familial breast cancer.

p53 tumour suppressor gene that controls cellular growth and differentiation is reported to be mutated in more than 50% of human cancers including breast cancer. p53 mutations are also known to increase the risk of breast cancer, as shown in Li-Fraumeni families. Recently, p53 gene particularly its exon 5 has been shown to be mutated in almost 100 of patients showing germline mutations of BRCA1.

Only exon 5 and exon 7 of p53 tumor suppressor gene have been screened for mutation in sporadic breast cancer cases. Out of 105 sporadic cases only three (2.8%) mutation could be detected in exon 5 of the p53 gene. No mutation was found in other exons. Out of 28 familial breast cancers analysed, only one mutation could be detected that too in exon 4 of the p53 gene.

4.5. Expression of BRCA1, BRCA2 and p53 genes in both sporadic and familial breast tumours.

For this study breast cancer biopsies collected on ice from surgical OT and immediately frozen in liquid nitrogen or –70°C and total cellular RNA and protein were extracted later by routine procedure. Samples were analyzed by northern blotting (slot blot) and western blotting. We analyzed 60 samples for m-RNA and protein expression of BRCA1 and p53 tumor suppressor gene. The results obtained so far indicate that the expression of BRCA1 gene is down-regulated while p53 gene is over-expressed in almost all breast cancer cases when compared to that of controls.

4.6. Role of promoter Methylation in the Expression of BRCA 1 Gene in Breast Cancer

DNA methylation is a universal reversible mechanism, which regulates gene expression, chromatin structure and genomic stability. Highly reduced expression of BRCA1 has been observed by us and others in sporadic as well as familial breast cancers although the actual mechanism(s) remains unclear. Mutation is often thought to be responsible for

BRCA1 inactivation in breast cancer but it does not affect the expression of BRCA1 gene. Aberrant cytosine methylation of the BRCA1 promoter is associated with decreased BRCA1 expression in human breast cancer. It suggests that epigenetic silencing may be one of the mechanisms of transcriptional inactivation of BRCA1 in sporadic breast cancer. Hypermethylation of the BRCA1 promoter region has been also strongly correlated with lack of estrogen and progesterone receptor expression. The role of abnormal methylation leading to loss of expression during carcinogenesis had already been established for several genes such as Rb, VHL, p15, p16, APC, E–cadherin, MGMT, GSTP1 etc. Present study has been carried out to see the status of methylation within the BRCA1 promoter region in a large spectrum of sporadic as well as familial breast cancer.

Investigation has been carried out after standardization of sodium bisulfite-based methods to analyze methylation in sporadic breast cancer. A total of 20 sporadic tumour biopsies were analysed so far. Only 3 cases (15%) showed hypermethylation of BRCA1 gene. Further study is in progress.

4.7. Role of Transcription factor AP-1, NFκB in sporadic and familial breast cancer with or without BRCA mutation.

Breast Cancer is one of the most common malignancies in women. Unfortunately, little is known about the specific molecular events, which cause the progressive transformation of human breast epithelial cells to malignant breast cancer. Various studies have revealed that multiple steps are involved in carcinogenesis including tumor ‘initiation’ and ‘promotion’ events. Mutations within tumor suppressor genes may represent the molecular equivalent of breast cancer ‘initiation’ events. However, the molecular mechanism of breast tumor ‘promotion is poorly defined. Also, the low rate of mutation as well as by low or absence expression of the

BRCA-1 and BRCA-2 genes (our unpublished data) indicates towards some probable regulatory events at the gene expression level. A key family of transcription factors, the activator protein AP-1 and pro-inflammatory protein NFκB has been shown to be associated with the development of breast cancer in different model systems.

4.8 Constitutive Activation of AP-1 and Increased NFkB p50/ p50 Homodimerization in Breast Cancer

Preliminary studies carried out in breast tumours specimen in comparision to that of normal controls indicate similar pattern of high binding activity of AP-1 in cancers. It also shows selectively high expression of c-fos and down-regulation of fra-1 in breast cancer. This is reconfirmed again in immunoblotting of AP-1 components using specific antibodies raised against each of AP-1 members.

5. MOLECULAR BIOLOGY OF ORAL CANCER

5.1 Molecular Biology of Oral Cancer

Squamous cell carcinoma of oral cavity is one of the most common malignant neoplasm in India. More than 4 lakhs cases are appearing every year. The disfiguring effect associated with significant mortality make the problem more alarming.

The contribution of tobacco and betel quid etc. is already documented as the major cause but recently the role of human papillomavirus (HPV) infection has been suggested by different investigating groups worldwide. Whatever may be the factor(s), it is gene(s) that are associated with cellular growth and differentiation must be affected as cancer in a sense is a genetic disease. Interplay of some host cell regulatory protein such as p53 and Rb with viral transforming genes E6, E7 are involved in deregulation of cell cycle leading to carcinogenic progression. Recently, the role of cellular inducible transcriptional factor such as AP-1 and NFκB in transcriptional regulation of viral oncogene expression has been suggested. Redox regulation of these genes by certain herbal (curcumin, neem) and synthetic (PDTC) anti-oxidants are also reported in cervical cancer. In oral precancer, curcumin has been specifically shown to have significant curative effects. However, molecular mechanism(s) underlying HPV-mediated oral carcinogenesis or its down regulation by antioxidants is not clear. It seems exciting and important to dissect molecular pathways involved during oral carcinogenesis with or without HPV infection.

With this background is mind following research activities have been initiated:

Detection and typing of HPV prevalence in oral precancer and cancer.

Analysis of expression of all members of transcriptional factors AP-1 and NFκB in oral cancer /precancer and their co-relation with cell lines derived from oral cancer.

Analysis of transcriptional regulation in HPV in oral premalignant and malignant tissues and oral cancer cell lines and its modulation by synthetic and herbal anti-oxidants.

6. HUMAN RESOURCE DEVELOPMENT

A. Ph.Ds Submitted

1. Uma Kailash: "Study of telomerase expression during development of cancer of the uterine cervix in women" Jawharlal Nehru Technological University, Hyderabad.

2. Suresh Hedau: "Molecular studies on the BRCA1 gene in breast cancer". Jamia Milia Islamia, New Delhi.

B. Ph.Ds Under registration

3. Neeraj Devendra Jain: "Molecular studies on the BRCA2 gene in breast cancer" Jamia Millia Islamia, New Delhi.

4. Ms.Nishat Jilani: “Role of hepatitis E virus (HEV) infection in acute and fulminant hepatitis during preganancy”, Jamia Millia Islamia, New Delhi.

5. Bhupesh K.Prusty: “Cellular Control of Human Papillomavirus Gene Activity”. Jamia Millia Islamia, New Delhi.

6. Alok Mishra: “Transcription control of Human Papillomvirus (HPV) oncogene expression in Oral Cancer”. Delhi University, Delhi.

7. Saket Chattapadhyay, “Study of Hepatitis B virus (HBV) Genotypes and their clinical significance in patients with HBV-related liver diseases”. Delhi University, Delhi.

8. Priyanka Verma , “Role of oxidative stress and cellular transcription factor AP-1 in pre and post operative cases of breast carcinoma", Delhi University, Delhi.

C. M.Ds awarded

1. Dr. Vikram Singh, Deptt. of Medicine, Lok Nayak Hospital, MAMC, New Delhi. “Analysis of p53 gene mutations in primary carcinoma lung and its relation to clinicopathological parameters” Delhi University, 2002.

2. Dr. Vishal Gupta : Department of Surgery, Lok Nayak Hospital, MAMC, New Delhi “The role of BRCA1, BRCA2 and p53 tumor suppressor genes in Breast and ovarian cancer” Delhi University, New Delhi, 2002.

3. Dr. Ritesh Sachdev, Department of Pathology, MAMC, New Delhi “p53 mutation in premalignant lesions of oral cavity and its relation to c-fos expression”, Delhi University, New Delhi, 2002.

4. Dr. Pralay Chakravarty, Department of Medicine, Lok Nayak Hospital, MAMC, New Delhi “Evaluation of hepatitis D infections in patients of hepatitis B related liver disease by PCR and genotyping of HDV isolates by RFLP”, Delhi University, Delhi, 2002.

D. M.D. Thesis submitted:

2002 -2003

1. Dr. Mingma L. Sherpa, Department of Biochemistry, Lady Hradinge Medical College and SSK Hospital, New Delhi, "Association of oxidative stress with c-fos and c-jun expression in breast cancer", Delhi University, Delhi, 2003.

2. Dr. Sudha Rani, Dept. of Obstetrics & Gynaccology, MAMC and Lok Nayak Hospital, New Delhi, "Evaluation of atypical squamous cells of undetermined significance (ASCUS) and low grade squamous intraepithelial lesions by detection of high risk HPV types with PCR", Delhi University, Delhi, 2003.

3. Dr. Arun Kundra, Deptt. of Medicine, MAMC & Lok Nayak Hospital, New Delhi. "A Prospective study to evaluate the role of new emerging hepatotropic viruses (Sen- V, TTV and HGV) in acute viral hepatitis and fulminant hepatic failure of unknown etiology", Delhi University, Delhi, 2003.

4. Dr. Tarun K. Bansal, Deptt. of Medicine, MAMC & Lok Nayak Hospital, New Delhi. "Detection of mutation in the precore region of the hepatitis B-virus genome in patients with hepatitis B related chronic liver disease and its clinical implication", Delhi University, Delhi, 2003.

5. Dr. Punam Gupta, Deptt. of Microbiology, MAMC, New Delhi. "Study of microbial biofilm with special reference to candida infection", Delhi University, Delhi, 2003.

6. Dr. Beneeta Kashyap, Deptt. of Microbiology, MAMC, New Delhi. "Role of mycoplasma pneumoniae in pediatric community-acquired lower respiratory tract infection", Delhi University, Delhi, 2003.

7. Dr. Ajay Yadav, Deptt. of Surgery, MAMC, New Delhi. "Transcription factor (Activator Protein-I) and microangiogenesis in breast cancer", Delhi University, Delhi, 2003.

DNB thesis submitted

8. Dr. Amita Gupta, DGO, Deptt of Gynae and Obstet., Lok Nayak Hospital, New Delhi. "Prevalence of HPV infection in male partners of women with cervical cancer." National Board of Examination, New Delhi, 2003.

DM thesis submitted

9. Dr. Brijesh, Deptt. of Medical Oncology, IRCH, AIIMS, New Delhi. "Telomerase activity and mRNA expression in acute lymphoid leukemia". AIIMS, New Delhi, 2003.

Special project Scientists Working:

1. CSIR Pool scientist – 2002-2005: Dr. Geetanjali, ENT Deptt, RML Hospital, New Delhi

2. DST sponsored Women Scientist (2003-2006): Dr. Shailja Pande.

M.SC. (BIOTECH) project Dissertations carried out. (For 4-6 Months): 2001-2002

1. Mr. Shailendra Yadav, "Role of NFkB during cervical carcinogenesis", Deptt. of Biotechnology, Purbanchal University, Jaunpur, 2002.

2. Ms. Hema Mohan, "Analysis of mutation and expression of breast cancer susceptibility gene BRCAl in breast cancer patients", Deptt. of Biotechnology, Kalicut University, Kalicut, Kerala, 2002.

3. Mr. Sinto Sebastian, "Study of telomerase mRNA expression in oral cancer and its clinicopathological significance", J.J. College of Arts and Science, Chennai, 2002.

4. Ms. Rachana Garg, "Role of AP-l transcription factor during oral carcinogenesis", School of Life Sciences, Devi Ahalya University, Indore, 2002.

5. Ms. Sharmistha Saha, "Expression of AP-l transcription factor in cervical cancer", Deptt of Biotechnology, Bundelkhand University, Jhansi, 2002.

6. Ms. Sherin Raja, “Role of NFkB in cervical cancer”, Department of Biosciences, Jamia Millia Islamia, New Delhi, 2003.

7. Ms. Preethi R, “Analysis of HPV 16 variants prevalent in women with cancer of the uterine cervix”, Department of Biotechnology, J.T. College, Pudukuttai, 2003.

8. Mr. Shashi Kant, “Role of NFkB in breast cancer”, Department of Zoology, H.N.B. Gharwal University, Srinagar, Uttaranchal, 2003.

9. Manpower development/ Training provided to:

Indian Science Academy Summer fellows:

1. Dr. K. Nataraja Seenivasan, Lecturer, PG & Research Department of Microbiology, K.S.R. College of Arts & Science, Tiruchegode – 637 209 - 2002

2. Ms. E. Manjuladevi. E, MBBS (final Year), Sri Sidhartha Medical College, Tumkur, Karnartaka – 2002

3. Mr. Ashok Kumar, M.Sc (Bio med. Genetics) PGI Basic Medical Science, Taramani, Chennai – 2003

4. Ms. Purabi Deka, M.Sc (Biotech.) Gauhati University, Assam - 2003

In Service and other Training:

1. Dr. Vinita Singh, RA, Institute of Pathology (ICMR), New Delhi – 110 002 (2002).
2. Mr. Trivikram Despande, Department of Zoology, Goa University, Goa (2002).
3. Mr. Md. Sahid, Department of Bioscience, Jamia Millia Islamia, New Delhi (2003).
4. Mr. Rajiv Vaid Basaiawmoit, School of Biotechnology, Madurai Kamraj Univerity, Madurai – 625 021(2002).

List of Summer Training Fellows, 2003

1. Kuhulika Bhalla, Sri Venkateswar College, University of Delhi, New Delhi.
2. Swadha Anand, Sri Venkateswar College, University of Delhi, New Delhi.
3. Deepali, MD University, Rohatak.
4. Nikhilesh S. Chand, St. Stephens College, New Delhi.

ICMR summer fellow

1. Dr.Manish K. Jha. MBBS 3rd year, MAMC, New Delhi

7. REFERRAL SERVICE

The Institute continued to offer referral services to professionals. During the year, referral services were offered as follows:

Cervical smears	882
Fine needle aspiration cytology	839
Non Gynae exoliative cytology	429
Histopathology	256

8. EXTRAMURAL PROJECT

1	Title	development of improved version of magnivisualizer
	Principal investigator	Dr.Aditya Parashari, Research Assistant
	Funding agency	Department of Science and Technology, Government of India(DST)
	Duration	3 years - 2000-2005
	Total budget	Rs.1,70,000
2	Title	Comparative role of genetic and environmental factors in the process of cervical carcinogensis
	Principal Investigator	Dr.A.B.Mitra, Dy.Director (Sr.Grade) & OIC
	Funding agency	DST
	Total funds	Rs.31.0 lakhs
	Duration	3 years - 2000-2003
3.	Title	Genetic alterations in adenocarcinoma of uterine cervix
	Principal Investigator	Dr.A.B.Mitra, Dy. Director (Sr.Grade) & OIC
	Funding agency	CSIR
	Total funds	12.73 lakhs
	Duration	3 years - 2000-2003
4.	Title	National workshop on Early detection of cervical cancer
	Principal Investigator	Dr.A.B.Mitra, Dy.Director (Sr.Grade) & OIC
	Funding agency	DST
	Total funds	Rs.4.0lakhs
	Duration	3 years - 2001-2003
5.	Title	Study of telomerase activity during development of cancer of the uterine cervix
	Principal Investigator	Dr.B.C.Das, Dy.Director (Sr.Grade)
	Funding agency	CSIR
	Total funds	Rs.13 lakhs
	Duration	3 years - 2000-2003
6.	Title	Transcriptional control of human apillomavirus oncogene expression in cervical cancer cells
	Principal Investigator	Dr.B.C.Das, Dy.Director (Sr.Grade)

	Funding agency	DBT
	Total funds	Rs.19 lakhs
	Duration	3 years - 2002-2005
7.	Title	Molecular Genetic basis of cancer: analysis of Genetic alteration and transcriptional profile of Genes during cervical carcinogensis
	Principal Investigator	Dr.B.C.Das, Dy.Director (Sr.Grade)
	Funding agency	ICMR
	Total funds	Rs.40lakhs
	Duration	3 years - 2003-2006
8.	Title	Study on the efficacy of Praneem cream and praneem polyherbal tablet on HPV infection of the cervix uteri: a pilot study
	Principal Investigator	Dr.B.C.Das, Dy.Director (Sr.Grade)
	Funding agency	ICMR
	Total funds	Rs.2lakhs
	Duration	2 years - 2002-2004
9.	Title	Development of DNA diagnostics for early detection of cervical cancer
	Principal Investigator	Dr.B.C.Das, Dy.Director (Sr.Grade)
	Funding agency	DBT
	Total funds	Rs.23 lakhs
	Duration	3 years - 2003-2006
10.	Title	Molecular markers for the detection and progression of cervical cancer
	Principal Investigator	Dr.B.C.Das, Dy.Director (Sr.Grade)
	Funding agency	DBT
	Total funds	Rs.23 lakhs
	Duration	3 years - 2003-2006
11.	Title	DST project on “Study of BRCA1 and BRCA2 Gene expression in human breast cancer cells”
	Principal Investigator	Dr.B.C.Das, Dy.Director (Sr.Grade)
	Funding agency	DST
	Total funds	Rs.19 lakhs
	Duration	3 years - 2003-2006
12.	Title	DST women scientist project on “Genetic polymorphism in E6 and E7 genes of Human Papillomavirus (HPV) types 16 and 18 in cervical cancer
	Principal Investigator	Dr.Shailaja Pandey under the supervisorship of Dr.B.C.Das, Dy.Director (Sr.grade)
	Funding agency	DST
	Total funds	Rs.17 lakhs

Duration	3 years - 2003-2006

9. LIST OF PUBLICATIONS

1. Murthy, NS: Reproductive Health Status of Indian Women- A Scenario; Obstetrics & Gynaecology-Today, 7,8,432-40,2002.

2. Murthy, NS. S.Sharma, A. Juneja, and A. B. Mitra: Potential years of life lost due to cancer mortality: Urban Experience, In: Bio Statistical aspects of Health and Epidemiology, (Eds.) Pandey CM, Mishra P, Singh U, Dept of Bio-Statistics, Sanjay Ghandhi Postgraduate Institute of Medical Sciences, Lucknow, India, 177-182, 2002.

3. Sardhana, S, Murthy NS, Sehgal A, Sharma S and Mitra AB: Cancers in women; Epidemiology, Health and Population, (Ed.) Anil Kumar, BR Publishing Corporation, Delhi, 223-234,2002.

4. Agarwal SS, Sehgal A, Roy M, Shashi S and Murthy N.S and Mitra AB: Feasibility of involving nursing students for early detection and prevention of cancers of uterine cervix and breast. Epidemiology, Health and Population, (Ed.) Anil Kumar, BR Publishing Corporation, Delhi. 235-240, 2002

5. Juneja A, Sehgal A, Agarwal SS, Murthy NS Singh V and Mitra AB. Selective cervical cytology screening for a developing country, Int J Gyn. & Obs. India, 5,4, 53-55,2002

6. Juneja A, Sehgal A, Agarwal SS, Singh V, Murthy N.S, and Mitra AB. A study of Obstetrics and Hygienic Practices in the development of High and Low grade lesions of the Uterine Cervix: Obstetrics & Gynaecology Today, 7, 9, 535-537, 2002.

7. Mathew A, Murthy NS and Sharma JB: Design of Randomised Clinical Trials, Obstetrics & Gynaecology Today, 8, 3, 131-138, 2003.

8. Juneja A and Murthy NS: Concept and relevance of “P” value in medical research, Obs & Gynae Today, 8 (6), 334-335, 2003.

9. Sharma S, Sardana S, and Murthy NS: Statistical software packages in Medical Research. Indian Association for Cancer Research- News Letter, 19 (1), 7-9, 2003

10. Puri J, Mishra B, Mala A, Murthy N.S, Thakur A, Dogra V, and Singh D; Catheter associated urinary tract infections in neurology and nuero surgicall units. J Infect. 44(3), 171-5, 2002.

11. Malhotra M, Sharma JB, Batra S, Sharma S, Murthy N.S, Arora R; Maternall and peri-natal outcome in varying degrees of anaemia; Inter. J Gynae. & Obstetric. 79, 2, 93-100, 2002.

12. Jain A, Rana SS, Chakravarty P, Gupta RK, Murthy NS, Nath MC, Gururaja S, Charturvedi N, Verma U, and Kar P: The prevalence of Hepatitis C virus antibodies among the voluntary blood donors of New Delhi, Eur. J Epidemiology, 18 (7): 695-7, 2003

13. Ray A, Naik SLD and Sharma BK (2002). Distribution of prognostically unfavourable product of c-erbB-2 oncogene and EGF-R in carcinomas of the breast and uterine cervix. *Indian J Physiol Pharmacol* 46: 423-433.

14. Ray A, Naik SLD and Sharma BK. Distribution of prognostically unfavourable product of c-erbB-2 oncogene and EGF-R in carcinomas of the breast and uterine cervix. Indian J Physiol Pharmacol 2002, 46: 423-33.

15. Bhambhani and Kashyp V: Fine needle aspiration (FNA) Cytology of thyroid in children and teenagers: a retrospective review. J Cytol 20 (I): 68-72, 2003.

16. Kashyap V and Bhambhani S. DNA aneuploidy in relation to tumor diathesis of cervical neoplasia. J Cytol 19(2) : 81-84, 2002.

17. Kashyap V & Bhambhani S. Co-existence of HPV infection with other lower genital tract infections in cervical smear. J Cytol 19(1) : 171-172, 2002.

18. Jain S, Kumar N, Sodhani P and Gupta S. Cytology of collagenous spherulosis of the breast: a diagnostic dilemma- report of three cases. Cytopathology 2002; 13: 116-120.

19. Gupta S, Sodhani P and Jain S. Macroconidia of Fusarium : an unusual finding in cervical smears. Acta Cytologica 2003; 47: 41-44.

20. Gupta S, Jain S. and Sodhani P. Alveolar soft part sarcoma: a rare entity with unique cytomorphological features. Cytopathology 2003; 14: 40-41.

21. Kumar N., Das P.M., Jain S., Sodhani P.and Gupta S. Melanoma of the soft parts: Diagnosis of metastatic and recurrent tumors by aspiration cytology. Diagn Cytopathol 2003; 28 : 295-300.

22. S. Garg, N. Sharma, P. Bhalla, R. Sahay, R. Saha, U. Raina, B.C Das, S. Sharma and N.S. Murthy : Reproductive morbidity in an Indian urban slum : need for health action. SEXUALLY TRANSMITTED INFECTIONS. 78 : 68-69, 2002.

23. Uma Kailash, S. Hedau, V. Gopalkrishna, S. Katiyar, and B.C. Das: A simple ‘paper smear’ method for easy collection, transport and storage of cervical specimens for PCR detection of HPV infection. J. MED. MICROBIOL. 51: 606-610, 2002.

24. S. Katiyar, B.K. Thelma, N.S. Murthy, S. Hedau, V. Gopalkrishna, N. Jain, S.A. Husain and B.C. Das: Polymorphism of the p53 codon 72 arg/pro and the risk of HPV type 16/18 associated cervical and oral cancer in India. MOLECULAR & CELLULAR BIOCHEMISTRY. 252: 117-124, 2003 .

25. B.C Das : Molecular biology techniques as applied to Cytology. J. OF CYTOLOGY. 20 : 15, 2003.

26. V. Singh, S. Salhan, B.C Das and A- Mittal : Predominance of Chlamydia trachomatis serovars associated with urogenital infections in females in New Delhi, India. J. CLINICAL MICROBIOLOGY. 41 : 2700-2702, 2003.

27. Naqvi SH, Saima W and Mitra AB. Restriction fragment length polymorphism of L1 amplicon using Rsa1 detectsfive different human Papillomavirus types and their co-infection among women attending a gynaecological outpatient department. Journal of Virological Methods. 117/1, 91-95, 2003.

28. Singh V, Sehgal A and Mitra AB. Concept of downstaging of cervical cancers. FOGCI FOCUS Preventive Oncology, Emerging trends of cancer 8-9; 2003.

Review articles and chapters in books:

1. Dr.B.C. Das, " Cellular control of Human Papillomavirus gene activity" in Proc. Ind. Natl. Sci. Acad-B, Vol.69: 23-34, 2003.

2. B.C. Das, Uma Kailash, Suresh Hedau, Neeraj Jain, Alok Mishra and Bhupesh K. Prusty: "Human Papillomavirus and Cervical Cancer." In Obst. & Gynae Today Vol. VIII : 347-353, 2003.

Participation in scientific, academic activities and conferences attended & papers presented –

Dr. NS Murthy

1) Temporary Advisor to the World Health Organisation for the meeting on "HPV Vaccine as a tool for prevention of Cervical Cancer" held at New Delhi, on 18-19th, September 2002 and delivered a talk on "Epidemiology of cervical pre-cancer".

2) Member of the Project Review Committee (PRC) for the division of NCD, ICMR, and attended several meetings at the ICMR Hqs during the year 2002-2003.

3) Member of the Multicentric National Task Force Group for the study on "Biology of HPV" and attended the meeting held at the ICMR Hqs, on 30th January, 2003.

4) Member of the Task Force group on Management of Cancers and attended the meeting at the ICMR Hqs on 26th February 2003.

5) Member of Screening Committee for screening of applications for the post of Research Officer, held at the ICMR Hqs on 17.3.2003

6) Elected member of National Editorial Advisory Board for the Journal Obs & Gynae -Today

7) Rendered advice on the design and analysis of data for the staff of ICPO relating to the research studies being carried out at the Institute and to a large number of researchers of Maulana Azad Medical College, Pant Hospital and other associated hospitals. .

8) Invited to deliver a lecture on the " Appropriateness of Cox Model over Logistic regression model for cohort data analysis" at the National Seminar on

applications of survival Analysis & Allied models held at National Institute of Mental Health and Neuro Sciences, Bangalore from June 21-22,2002.

9) Invited to deliver a series of lectures in Statistics for the Post Graduate students in the Dept. of Psychiatry, GB Pant Hospital, New Delhi from 2 July to 12th July' 2002.

10) Invited to deliver a lectures on " Statistics in Obstetrics and Gynaecology" for the Post Graduate students in the Department of Obstetrics and Gynaecology at MAMC, New Delhi on 23-7-2002.

11) Co-Supervisor for Mr. Mohammad Shahid, working for his Ph.D at the Jamia Millia Islamia, and Dr. Neeraj Gupta, Student of MD Psychiatry, at GB Pant Hospital, New Delhi.

12) Reviewer for the various bio medical journals.

13) Attended the 20th Annual meeting of Indian Society for Medical Statistics held at Institute for Research in Medical Statistics, New Delhi, from 19-22, Dec. 2002.

14) Attended the 22nd Annual Convention of Indian Association for Cancer Research and International Symposium on Recent Advances in Cancer Causes and Control held at the Regional Cancer Centre, Thiruvananthapuram, from 10-12, January, 2002 and presented a paper entitled " An indicator for determining priority for cancer control activities in Indian situation".

Dr.B.C.Das

A. As chairman of Conferences

1. Chairman of Scientific session on “genetic markers for malignancies” in Medicine update, Maulana Azad Medical College, New Delhi, December 18, 2002.

2, Chairman of Scientific session on “HPV and Cancer” at the 22nd Annual Convention and International Symposium on Recent Advances in Cancer Causes & Control. Regional Cancer Centre, Trivandrum, January 10-12, 2003.

3. Chairman of Scientific session on “Environmental Toxicology, Mutagenicity and Human Health XXVII EMSI Annual Conference, Indian Institute of Chemical Biology, Kolkata, February 14-16, 2003.

B. Delivered/Invited plenary talks on

1. “HPV and Cervical Cancer” at Railway cancer Hospital, Varanasi, April 3, 2002.
2. “Molecular Medicine: Lab to Land” in CME - Molecular Medicine. Lady Hardinge Med. College, New Delhi, August 10, 2002.
3. “Molecular tools in the diagnosis of hepatitis C” in CME: Gastroentology: Deptt. Of Medicine, MAMC, New Delhi, September 1, 2002.
4. “Molecular tools in the diagnosis and management of HPV and cervical cancer” in WHO/ICMR Workshop on HPV Vaccine as a tool for prevention of cervical cancer, The Oberoi Hotel, New Delhi, September 18-19, 2002.
5. “Role of transcription factor AP-1 in oral cancer” in 2nd World Assembly on Tobacco Counters Health (WATCH-2002), Taj convention center, New Delhi, September 29-October 3, 2002.
6. “Molecular Biology Techniques as applied to cytology” in symposium on “Ancillary Techniques as applied to cytology.” Annual conference of Indian Academy of Cytologists PGIMS, Chandigarh, December 1, 2002.
7. “Molecular Diagnosis of Co-infections” in 11th Round Table Conference on “Hepatitis B and Co-infections” of Ranbaxy Science Foundation India Habitat Centre, New Delhi, December 3, 2002.
8. “Tumorigenicity of cervical cells correlates with alteration in Fos gene expression” in 22nd Annual convention of Indian Association for Cancer Research. Regional Cancer Centre, Trivandrum, January 10-12, 2003.
9. “The role of BRCA1 and BRCA2 gene mutation in Indian women with sporadic and familial breast cancer” in XXVII Annual Conference of EMSI, Indian Institute of Chemical Biology, Kolkata, February 14-16, 2003.
10. “Transcriptional control of Human Papillomavirus infection” in Mid year meeting of Indian Academy of Sciences, Bangalore, July 18-20, 2003.
11. As WHO expert attended
12. meeting on “International collaborative studies on HPV reagents for Laboratory Diagnostic Procedures” WHO, Geneva, September 24-25, 2003.

Dr.Pushpa Sodhani

1. 32nd Annual Conference of Indian Academy of Cytologists at PGI, Chandigarh 28th Nov-1st Dec. Presented a paper "Bethesda system of reporting: experience in a hospital based screening programme for early detection of cervical cancer"

2. 51st Annual Conference of Indian Association of Pathologists and Microbiologists at Kotlkata 13th-15th Dec. 2002. Presented a paper on Cytomorphology of alveolar soft part sarcoma.

3. Attended quarterly Delhi Chapter meetings of IAPM

4. Attended Breast group meetings

Dr.Sanjay Gupta

1. 32nd Annual Conference of Indian Academy of Cytologists at PGI, Chandigarh 28th Nov-1st Dec. Presented a paper "Rapid rescreening of cervical smears: a recent adjunct to quality control in cervical cytology"

2. 8th International CME on surgical pathology and Ramalingaswami Symposium, at AIIMS, Delhi 12th-15th Feb, 2003.

3. Attended quarterly Delhi Chapter meetings of IAPM

Dr.Suresh Bhambhani

1. Co-supervisor for MD/MS thesis. A comparison of fine needle aspiration cytology with smear for the diagnosis of carcinoma cervix. Maulana Azad Medical College, Deptt. Of Obs & Gynae 1999-2002.

2. Invited by WHO as temporary advisor for WHO/ICMR workshop on HPV vaccine as a tool for prevention on cervical cancer. Delivered lecture on "diagnostic methods for cervical cancer and pre-cancer in India" N.Delhi 18-19 Sept 2002

3. Appointed as expert for imparting training in cytology and early detection of cancer for cytotechnicians and cytopathologists sponsored by WHO. Bikaner Medical College Dec 24th-Jan 2nd 2003.

4. Chaired the session on proferred papers at annual conference of Indian Academy of Cytologists, PGI Chandigarh-30th Nov. 2002.

CAMP

Participated in a cancer detection camp organized by East Delhi Gynaecologists Forum on 8th Dec. 2003 with a focus on early detection of cancers of uterine cervix and breast. A total of 1833 cervical smears were screened and diagnosed by us and the epithelial cell abnormalities detected (1.7%) were referred for further management and follow up.

11. LIST OF MEMBERS OF ETHICAL COMMITTEE

MEMBERS OF THE ETHICAL COMMITTEE MEETING OF ICPO HELD ON 6TH

JUNE,2001.

1. Dr. S.C. Jain,
   Member Secretary,
   Law Commission & Secy to the Govt. of India, Chairman
   Room No. 730-A,
   Shastri Bhawan,
   New Delhi-110001.

2. Dr. K.D. Tripathi,
   Director Professor,
   Department of Pharmacology,
   Maulana Azad Medical College,
   New Delhi-110002.

3. Dr. K.K. Pandey,
   Senior Consultant,
   Deptt. Of Surgery,
   Rajiv Gandhi Cancer Institute & Research Centre,
   Sector V, Rohini,
   Delhi-110085.

4. Dr. Kishore Chaudhry,
   Dy. Director General,
   ICMR, New Delhi.

5. Dr. V.L. Bhargava,
   Sr.Consultant (Gynae),
   Sita Ram Bhartiya Institute & Research Centre,
   B-16, Mehrauli Institutional Area,
   New Delhi-110026.

6. Dr. A.B. Mitra,
   Officer-In-Charge,
   Institute of Cytology & Preventive Oncology, Member Secy.
   MAMC Campus,
   New Delhi-110002.

12. LIST OF MEMBERS OF THE SAC

List of Scientific Advisory Committee Meeting of the Institute to be held on 30th Dec,2002 at ICPO.

1. Prof. N.K. Ganguly,
   Director General,
   ICMR, New Delhi.

2. Dr. Padam Singh,
   Addl. Director General,
   ICMR, New Delhi.

3. Dr. Bela Shah,
   Sr.DDG(NCD),
   ICMR, New Delhi.

4. Prof. N.C. Nayak
   X-29, Hauz Khas, Chairman
   New Delhi.

5. Dr. A.N. Bhisey,
   7, Yugprabhat Co.Op.Housing Society,
   OPP. Sitladevi Temple Road,
   Mahim,
   Mumbai-400 016.

6. Dr. S.K. Basu
   Director,
   National Instt. Of Immunology,
   Shaheed Jeet Singh Marg,
   New Delhi-110067.

7. Dr. Sunita Saxena,
   OIC, Instt. Of Pathology,
   Safdarjung Hospital Campus,
   New Delhi-110029.

8. Dr. Kamala Krishnaswamy,
   SRINIKETAN,
   10-C, Block-B, 2-98/2,
   Habsiguda,
   Hyderabad-500 007.

9. Prof. G.K. Rath,
   Head, Deptt. Of Radiation Therapy,
   Rotary Cancer Institute,
   AIIMS, Ansari Nagar,
   New Delhi-110029.

10. Dr. Babu Mathew,
Regional Cancer Centre,
P.O. Box No. 2417,
Medical College Campus,
Thiruvananthapuram-695011

11. Dr. N.K. Mehra,
Deptt. Of Histocompatibility &
Immunogenetics,
AIIMS, New Delhi.

12. Dr. Paratha Sarathi Basu,
Chittaranjan National Cancer Institute,
37, S.P. Mukherjee Road,
Kolkata-700026

13. Dr. D. Takkar,
Formerly Prof. & Head,
Deptt. Of Gynae,
AIIMS

14. Prof. Arati Bhatia,
Prof. Of Cytopathology,
UCMS & GTB Hospital,
Shahdra , Delhi.

15. Dr. R.N. Gupta,
Scientist Emeritus
ICMR, New Delhi.

16. Prof. Subrata Sinha,
Deptt. Of Biochemistry,
AIIMS, New Delhi.

17. Dr. Shahid Jameel,
Chief,
Virology Group (ICGEB),
Aruna Asaf Ali Marg,
New Delhi-110067.

18. Dr. B.K.Dhaon
Dean, MAMC,
New Delhi.

19. Dr. A.N. Sinha,
Deputy Director (NCD),
Directorate General of Health Services,
Nirman Bhavan,
New Delhi-110011.

20. Dr. R.K. Navlakha,
Medical Supdt.,
Lok Nayak Hospital,
New Delhi-110002.

21. Prof. Subhash K. Gupta,
Professor and Head,
Deptt. Of Cytology & Gynae. Pathology,
Post Graduate Institute of Medical
Education and Research,
Chandigarh-160012.

22. Dr. A.B. Mitra,
Officer-In-Charge,
ICPO, New Delhi. Member Secretary

13. STAFF

OFFICER-IN-CHARGE

Dr.A.B.Mitra, M.Sc., Ph.D. Dy.Director (Sr.Grade)

DIVISION OF CYTOPATHOLOGY

DEPUTY DIRECTOR
Dr.Pushpa Sodhani, M.B.B.S., M.D.(Path)

ASSISTANT DIRECTOR
Dr.Sanjay Gupta, M.B.B.S., M.D.(Path.)

RESEARCH OFFICER
Dr .Malasha Kumari, MBBS

CYTOTECHNOLOGIST
Mr.K.L.Chachra, M.Sc(M.L.T)

TECHNICAL OFFICER
Mr.J.N.Pant, B.A., Dip (M.L.T) Retired on 31st July, 2003
Mr.I.U.Khan, Matric, Dip.(M.L.T) Retired on 31st March , 2003

SR. TECHNICAL ASSISTANT
Mrs. Vijay Khan, B.Sc. Dip. (M.L. T)

CYTOTECHNICIAN
Mr.N.C.Bhatt, B.Sc (M.L.T)

LAB TECHNICIAN
Mr.Sham Sunder

HUMAN RESOURCE DEVELOPMENT SECTION (CYTOPATHOLOGY)

DEPUTY DIRECTOR (Sr. Grade)
Dr.Suresh Bhambhani, M.B.B.S., M.D. (Path),

SENIOR RESEARCH OFFICER
Dr.Veena Kashyap, M.Sc(Zool), Ph.D.

LABORATORY TECHNICIAN
Miss.Pushpa Bhadola, M.Com., M.L. T .

DIVISION MOLECULAR GENETICS

DEPUTY DIRECTOR (Sr.Grade)
Dr.A.B.Mitra, M.Sc(Zool), Ph.D

ASSISTANT DIRECTOR
Dr. Mausami Baradwaj w.e.f 1.8.2003

SR.RESEARCH OFFICER
Mr.J.K.Sharrna, M.Sc(Hum Genet) M.Phil(Anthropology)

RESEARCH OFFICER
Mr. R. Suresh Kumar, M.Sc.
SR. TECHNICAL ASSISTANT
Dr.Anita Sharma, M.Sc (Hum Genet) M.Phil, Ph.D
TECHNICAL ASSISTANT
Mr.Mohinder Pratap, M.Sc.,
LABORATORY TECHNICIAN
Mrs.Thankamma James, Matric (M.L.T.)
SENIOR RESEARCH FELLOW
Samar Husain Naqvi
Saima Wajid

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