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-: 1 :-
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 precancerous 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 precancancerous 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 dimerization 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 concentration 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 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 preneoplastic 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 (GST M1 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.