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:

<table>
<thead>
<tr>
<th>Category</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Within normal limits</td>
<td>711</td>
</tr>
<tr>
<td>Benign cellular changes</td>
<td>410</td>
</tr>
<tr>
<td>Epithelial cell abnormalities</td>
<td>43</td>
</tr>
<tr>
<td>ASCUS</td>
<td>29</td>
</tr>
<tr>
<td>AGUS</td>
<td>1</td>
</tr>
<tr>
<td>LSIL</td>
<td>6</td>
</tr>
<tr>
<td>HSIL</td>
<td>4</td>
</tr>
<tr>
<td>Malignant</td>
<td>3</td>
</tr>
</tbody>
</table>

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:

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

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 herbal 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 cancer.

\[ a) \text{ Increased DNA binding activity and differential expression pattern of AP-1 transcription factor in cervical cancer tissues.} \]

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 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.

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 gynaecological 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, E7 and 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 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.

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 polymorphic markers for the chromosomal arms 3p (3p14.1-14.2) and 5p(5p15.1-15.2) for their reported high LOH rate.

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

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)

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

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
c 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

GSTM1 null and GSTT1 null genotypes in cancer and controls