SUMMARY AND CONCLUSIONS
CHAPTER 6
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In the present study on 125 cases of squamous cell cervical carcinoma, 92.8% of the patients were positive for HPV.

Methylation of \(p15^{\text{INK4B}}\) (p-value<0.05) and \(p16^{\text{INK4A}}\) (p-value<0.001) were observed to be significant in the population under study but \(p14^{\text{ARF}}\) methylation was not found to be significant in the present population (p-value>0.05). The hypermethylation of \(p16^{\text{INK4A}}\) significantly increased the risk of cervical cancer. The hypermethylation of \(p15^{\text{INK4B}}\) showed marginal association with the risk of cervical cancer. Hypermethylation of \(p15^{\text{INK4B}}\) was not observed to show significant correlation with the risk factors. Hypermethylation of \(p16^{\text{INK4A}}\) was observed to have significant association with passive smoking (p-value<0.05) and use of oral contraceptives (p-value<0.001). However, statistically significant correlation was not observed with HPV infection. \(p16^{\text{INK4A}}\) methylation was not found to be age-dependent.

It was also not affected by AFSI. Hypermethylation of \(p16^{\text{INK4A}}\) promoter significantly decreased the mRNA expression in patients in comparison to patients with unmethylated \(p16^{\text{INK4A}}\) promoter (p-value<0.001). Combination of methylated \(p14^{\text{ARF}}\) and methylated \(p16^{\text{INK4A}}\) was found to have a significant correlation with the increase in the risk of cervical cancer with respect to OC users, age>45 years and AFSI>20 years (p-value<0.05).

No significant association between hypermethylation of \(p21^{\text{CIP1}}, p27^{\text{KIP1}}, p57^{\text{KIP2}}\) and the risk of cervical cancer was observed in the present study (p-value>0.05). During the analysis of hypermethylation of these genes, statistically significant correlation was not observed with HPV infection, age group, passive smoking, use of oral contraceptives and AFSI with respect to the increase in the risk of cervical cancer.

\(p53\) hypermethylation was found to be significant in cervical cancer patients in the present population (p-value<0.05). Statistically significant correlation between hypermethylation of \(p53\) and increase in the risk of cervical cancer with respect to HPV infection and passive smoking was not observed. Age was not a factor for increase in the risk of cervical cancer with respect to hypermethylation of \(p53\) gene in the present population of study.
Significant increase in risk of cervical cancer was observed with respect to p53 hypermethylation in OC users and patients with AFSI<20 years (p value<0.05).

There was significant association between hypermethylation of p73 and the risk of cervical cancer in present study. Hypermethylation of p73 was found to be statistically insignificant to HPV infection and OC use but significant association with the increase in the risk of cervical cancer was observed with respect to passive smoking (p-value<0.05). Age and AFSI were not observed to have any impact on p73 hypermethylation in the present study. Hypermethylation of p73 promoter significantly decreased the mRNA expression in patients carrying methylated promoter as compared to patients with unmethylated promoter (p-value<0.001). Combination of p53 and p73, when both the genes were methylated together, in correlation with passive smokers, OC users, patients with age>45 years and AFSI did not significantly increase the risk of cervical cancer in the present population of study.

A strong association between hypermethylation of RARβ2 and the risk of cervical cancer was observed. Hypermethylation of RARβ2 was observed to have significant correlation with increase in the risk of cervical cancer in association with passive smoking and use of oral contraceptives (p value<0.001). RARβ2 methylation was not found to be age-dependent. It was also not affected by AFSI. Hypermethylation of RARβ2 significantly decreased the mRNA expression in patients as compared to patients with unmethylated promoter (p value< 0.001).

There was significant association between hypermethylation of FHIT and DAPK and the risk of cervical cancer in the present population of study (p value<0.001). Hypermethylation of these genes showed no significance with respect to HPV infection. Hypermethylation of FHIT and DAPK was found to be significantly increasing the risk of cervical cancer with respect to passive smoking and use of oral contraceptives (p value< 0.001). However, age and AFSI were not observed to have much impact on the hypermethylation of these genes in the present study. Hypermethylation of promoters of FHIT and DAPK appreciably decreased the gene expression in patients as compared to patients with unmethylated promoter for the same gene (p value< 0.001).
No significant association of hypermethylation of \(RB1\) and \(STAT1\) was observed with the risk of cervical cancer in the population under study.

In the present study, promoter hypermethylation was found to increase with the stage of cervical cancer in case of \(p16\). However, more number of cases need to be studied for each stage to draw a statistical significance.

A significantly increasing trend for methylation of \(p73\), \(RAR\beta2\), \(FHIT\) and \(DAPK\) genes was observed with the stage of cervical cancer. The \(\gamma\)-coefficient was also found to be significant for these genes.

According to the results of the present study, promoter hypermethylation was detectable in serum samples of cervical cancer patients with high sensitivity of 83.3%.

Since, epigenetic changes are reversible, treatment of SiHa cells with genistein and curcumin (20 \(\mu\)M each) showed reversal of \(RAR\beta2\) gene after 72 h and this demethylation was observed to increase as the time period of treatment was increased to 6 days. The expression of mRNA increased proportionally with the appearance of unmethylated DNA. Genistein was more effective in causing the reversal of promoter hypermethylation. EGCG was also observed to cause significant reversal of hypermethylation of \(RAR\beta2\) gene after 72 h of treatment and this demethylation was observed to increase as the time period of treatment was increased to 6 days. Curcumin, genistein and EGCG resulted in demethylation of \(RAR\beta2\) gene and reactivation of \(RAR\beta2\) gene even in HeLa cell line.

Other dietary compounds like capsaicin, resveratrol and ethanolic extracts of \(Ocimum sanctum\), \(Withania somnifera\) and \(Azadirachta indica\) did not result in any reversal of hypermethylation of \(RAR\beta2\) gene till 72 h of treatment in SiHa and HeLa cell lines...