Summary

Oral squamous cell carcinoma (OSCC) and esophageal squamous cell carcinoma (ESCC) develops as a result of complex epigenetic, genetic and environmental interactions. Epigenetic changes like, promoter hypermethylation of multiple tumour suppressor genes are very crucial and frequent events in cancer. It is assumed that certain habit-related carcinogens and viral infections are capable of inducing aberrant methylation responsible for initiation and progression of cancer. However, the effects of environmental carcinogens depend upon the level of metabolism by carcinogen metabolizing enzymes and their possible involvement in modulating DNA methylation is clearly known. Moreover, Human papillomavirus (HPV) is being associated with squamous cell carcinoma of oral cavity and esophagus in recent few years but its possible role in promoting aberrant methylation in these tumours has largely remained unexplored. Here, we studied the interaction of various habits related factors and polymorphism of \( \text{GSTM1} \) and \( \text{GSTT1} \) genes towards inducing promoter hypermethylation of multiple tumour suppressor genes. We also investigated the association of HPV with aberrant methylation in tumour-related genes/loci consisting of the classical CpG Island Methylator Phenotype (CIMP) panel markers (\( p16, \text{MLH1}, \text{MINT1}, \text{MINT2} \) and \( \text{MINT31} \)) and other frequently methylated cancer-related genes (\( \text{DAPK1, GSTP1, BRCA1, Ecad} \) and \( \text{RASSF1} \)) and survival of OSCC and ESCC.

The study included 62 OSCC, 112 ESCC cases and 130 age and gender matched controls. Conditional logistic regression was used to calculate odds ratios (OR) and multifactor dimensionality reduction (MDR) was used to explore high order interactions. Detection of HPV and aberrant promoter methylation was performed by PCR and Methylation Specific PCR respectively. Association study was conducted by Logistic regression analysis and overall survival analysis was done by Kaplan-Meier plot.
Major Finding:

- Tobacco consumption (both chewing and smoking) was found significantly associated with more than 2-folds increased risk of OSCC and ESCC. Moreover, betel-quid chewing was associated with almost 3 folds increase in risk of OSCC.
- Individuals with GSTM1 and GSTT1 null genotypes were associated with an increased risk of OSCC and ESCC respectively and the interaction of tobacco chewing /betel quid chewing/ smoking with GSTM1 or GSTT1 was predicted to the best model for OSCC and ESCC development respectively, based on MDR analysis.
- Nearly ~30% of OSCC and ESCC patients were classified as CIMP-high and DAPK1 and RASSF1 promoters were the most frequently methylated genes with ~50% methylation frequency. In addition, methylations of classical CIMP panel markers were significantly correlated with methylation status of other TSGs under study, designating the efficiency of classical CIMP panel in OSCC and ESCC.
- Tobacco (chewing & smoking) is highly associated with CIMP in OSCC and ESCC with OR nearly 6.67 for tobacco chewing and 2.64 for smoking respectively.
- CIMP-high cases showed poorer survival than CIMP-low cases. Moreover, promoter hypermethylation of p16 and DAPK genes were significantly associated with poorer survival.
- ~36% of the patients were HPV (+) and a surprisingly higher percentage of HPV-18. However, HPV presence was significantly associated with better survival in OSCC but not in ESCC patients.
- Around 70% of CIMP high tumours were associated with HPV presence and higher methylation frequency of all the loci consequently exhibiting higher methylation index.
- Hierarchal cluster analysis revealed three distinct tumour sub-groups based on promoter methylation of the 10 genes/loci. Among these, a CIMP-high subgroup was identified, associated with highest proportion of HPV presence, highest mean methylation index and highest tobacco consumption.