Head and neck cancer (HNC) is the sixth most common and seventh leading cause of cancer- related deaths [1] with approximately 650,000 new cases and 350,000 deaths per year worldwide [2]. Several reports have indicated wide variation in the incidences of HNC across the world, with highest prevalence in the Southeast Asia. In India, HNC accounts for 30–40% of cancers compared to approximately 9% in Taiwan and 2-4% in western countries [3]. The prevalence of HNC is reported to be highly significantly at 54.48% and 33% of tobacco related oral cancer in Northeast, India. [4]. The crucial risk factors are tobacco, alcohol consumption, betel nut chewing, and HPV infection for the development of HNC [5]. Betel nut/betel quid chewing also accompanies to the development of esophageal, oropharyngeal and oral cancer [6,7].

In our thesis it is demonstrated that chewing of betel nut enhanced the risk of HNC in combination with tobacco chewing. In the NE population of India, betel nut (BN) with lime is traditionally consumed by the people as part of their cultural habits. BN contains raw or fermented areca nut commonly known as 'tamul' and small portion of betel leaf with or without any other constituents and is used frequently [8].

Furthermore, genetic polymorphism in xenobiotic metabolizing and DNA repair enzymes modifies the effect of environmental exposure to carcinogen factor and plays a significant role in gene-environment interaction and hence contributing to the individual susceptibility to cancer. The present study emphasis on interaction between the genetic polymorphism and environment exposer to carcinogen. Xenobiotic metabolizing genes and DNA repair genes suggest the susceptibility markers and genotype profiles assigning to increased risk and HPV infection associated with HNC.

The present thesis was conducted with the aim of study is to establish a relation between genetic polymorphism (caused by consumption of betel nut, tobacco and alcohol) caused by cancer development and detection of HPV infection with p16 expression status in HNC tumor tissue and their correlation with tumor stages, betel nut, tobacco, smoking and alcohol consumption serve as biomarker and diagnosis of HNC.

Based on the 205 histopathologically confirmed cases and 210 healthy controls. We could conclude the CYPs, GSTs, EPHX1, NAT1, NAT2, ADH, ALDH, XRCC1, XPD and MGMT and risk of dietary habits. The following observations were made:

Polymorphic CYPs and GSTs genes and HNC risk

We investigated association of HNC with gene variants involved in detoxification i.e. CYP1A1, CYP2E1, GST P1, M1 and T1 and risk of dietary habits.

- CYP1A1 and CYP2E1 homozygous having 3.41 and 6.59 fold increased risk in betel nut chewers.
- CYP1A1 homozygous having 5.09 increased fold and CYP2E1, 10.23 fold risk in tobacco chewers.
- Among GSTs, GSTT1 null showing increased risk 1.73 fold in betel nut chewers, 2.86 fold in tobacco chewers and 2.39 fold in smoking habits
- The synergetic effect of betel nut, tobacco chewing and smoking habits in CYPE1 variants 10.43 fold and increased risk of GST T1 null 7.22 fold when compared with betel nut and tobacco chewing alone.
- There was an interaction between HNC and CYP2E1, GST T1 null genotype that increase the risk with betel nut, tobacco and smoking habits.

Polymorphic EPHX1, NAT1 and NAT2 genes and HNC risk

We investigate the distribution of variant EPHX1, NAT1 and NAT2 polymorphism and combination of haplotype status with environmental exposures (betel nut, tobacco, and smoking) and to evaluate their association with head and neck carcinogenesis in NE Indian population.

- We observed the EPHX113 (CC) genotype with exposure of betel nut (3.37 fold), tobacco (3.85 fold) and betel nut-tobacco (3.45 fold) is found to have increased risk for HNC.
- EPHX139 (AG) genotype with exposure of betel nut (3.42 fold), smoking (3.12 fold), and betel nut-tobacco- smoking (5.22 fold) is found to have increased risk for HNC.
- The TC and CC genotype of EPHX113 increase the risk for HNC in betel nut (both doses) and betel nut-tobacco (both dose of tobacco).
- The AG genotype of EPHX139 increases the risk in betel nut only (lower dose) and betel nut-tobacco (both dose of tobacco) cases.

- The NAT2 C481T (CT and TT) was found to increase HNC risk association with only betel nut, only tobacco and smoking habits.
- The NAT2 G590A (AA) genotype increases the risk only in association with smoking habits.
- All the doses of betel nut and tobacco were found to have strong impact on HNC risk in all genotypes of NAT2 C481T (except TT) and all genotypes of NAT2 G857A (except AA).

Polymorphic ADH2, ADH3 and ALDH2 genes and HNC risk

We have also investigated distribution of ADH2, ADH3 and ALDH2 genotypes and their interactions with betel nut, tobacco and risk stratified by drinking status.

- Our case-control study demonstrated that higher consumption of alcohol increased the risk for HNC 4.40 and 3.28 fold in the BN and BN–tobacco cases respectively but lower doses were found to be protective.
- The risk alleles of ADH2 and ADH3 had strong impact and in combination exhibited synergistic role on the HNC risk in the NE subjects.
- Our study further demonstrated that the risk ADH2*1/*2 allele increased 3.49 and 3.39 fold risk of HNC in BN and BN-tobacco associated HNC.
- The risk allele ALDH2*1/*2 or ALDH2*/*2 had impact on the risk for HNC interaction with BN, tobacco and alcohol drinking status
- The higher intake of alcohol synergistically increased 5.35 fold risk in BN-tobacco cases as compared to BN case alone.
- Our study is the first report that identified the risk of higher consumption of alcohol with risk alleles of ADH2 and ALDH2 on the risk for BN and BN - tobacco associated HNC in the NE population of India.

Polymorphic XRCC1, XPD and MGMT and HNC risk

We investigated the prevalence of HNC cases with deciphering the role of XRCC1, XPD and MGMT, their interaction with intake of betel nut chewing, tobacco, smoking and alcohol consumption of HNC patients in NE region

- The XRCC1 Arg194Trp with heterozygous CT (1.68 fold) and mutant TT (3.58); XPD with heterozygous CA (1.70 fold) and mutant AA (3.56 fold) genotypes were found to be increases risk for HNC.
- The MGMT (Trp65Cys and Leu84Phe) gene did not show any risk of HNC in the North East Indian Population.
- The CT and TT genotype of XRCC1 was found to increases the risk for HNC in cases with betel nut alone (both dose), betel nut and tobacco (both dose of tobacco), betel nut and alcohol (both dose of alcohol).
- The CT genotype of XRCC1 was found to increase the risk for HNC in betel nut - alcohol (higher dose of alcohol).
- The CA, AA genotype of XPD increases the risk for HNC in only betel nut (both dose), betel nut and tobacco (both dose of tobacco), betel nut and alcohol (higher dose of alcohol) and betel nut and tobacco and alcohol (higher dose of alcohol, CC is exception) cases.
- The CA genotype of XPD increases the risk for HNC in betel nut and tobacco and alcohol (lower dose of alcohol) cases.
- The lower dose of betel nut (in only betel nut) and tobacco (in betel nut and tobacco) increases the risk for HNC in patients with CT, TT and CT genotype of MGMT cases respectively.
- In the higher dose of (betel nut- alcohol) increases the risk for HNC in patients with CT genotype of MGMT gene.

HPV status with p16 expression and HNC risk

Human papilloma virus (HPV) associated Head and Neck Cancers (HNCs) have generated significant amount of research interest in recent times. Due to high incidence of HNCs and lack of sufficient data on high-risk HPV (hr-HPV) infection from North -East region of India, this study was conceived to investigate hr-HPV infection, its types and its association with life style habits such as tobacco, alcohol consumption etc.

- A total 106 HNC case to determine causative role of HPV.
- It was observed the presence of hr-HPV was confirmed in 31.13% (n = 33) of the HNC patients by nested multiplex PCR (NMPCR) and HC2 assay respectively.

- Among hr-HPV positive cases, out of thirteen hr- HPV types analyzed, only two prevalent genotypes, HPV- 16 (81.81%) followed by HPV-18 (18.18%) were found.
- Significant association was observed between hr-HPV infection with alcohol consumption ($p < 0.001$) and tobacco chewing ($p = 0.02$) in HNC cases. Compared to HPV-18 infection the HPV-16 was found to be significantly associated with tobacco chewing ($p = 0.02$) habit.
- To confirm the biologically active HPVs, p16 expression was analyzed in both HPV positive and HPV negative cases, and over expression of p16 was found in HPV positive cases which is an indirect indicator for the presence of HPV.
- Our study demonstrated that tobacco chewing and alcohol consumption may act as risk factors for hr-HPV infection in HNCs from the North East region of India.

Conclusion and Future prospects

Understanding the genetic alterations in patients from particular population would help in better understanding of the disease prognosis and will be useful in predicting the severity of the disease. These polymorphic status of the genes and HPV status might serve as possible predictive biomarkers for early detection of HNC. Furthermore, gene-gene and gene-environment interaction between the genetic polymorphism and prospective life style risk factor in particular population would be instrumental in determining the preventable life style factors in order to reduce the burden of HNC. Furthermore, the polymorphic status of particular genes in specific population will provide insight into the development of personalized effective therapeutic regimens based on pharmacogenomics principle for better management of disease severity and treatment.