Abstract

Cancer has emerged as a second leading cause of disease related death worldwide accounting 8.2 million deaths and 14.1 million new cases in 2012. Despite of huge stride made in diagnostic and therapeutic, the incidence, prevalence and mortality associates with cancer has been increasing day by day. Report estimated that, in India head and neck cancers (HNC) account for 30-40% of total body malignancy (TBM) [2] with ~0.15 million newly diagnosed cases, ~0.11 million death and 0.24 million prevalence in 2012. In North East part of India, the prevalence of head and neck cancers are very high particularly oropharynx carcinoma is higher among the other HNC. The prevalence is found to be significantly high at 54.48%, affecting males more than females in the age group of 40–69 years.

Many factors are implicated in development of this pathological state including smoking habits, tobacco use, chewing of betel nut/areca nut, alcohol consumption, dietary habit etc. Some of these habits are associated with the socio-cultural aspects of the region. Allelic variations in individual or in population also can have profound effect on predisposition of particular disease. Recently, work on individual variation and its relevance as a predisposition factor for cancer have been gaining importance. Genetic polymorphisms in genes controlling carcinogen metabolism, DNA repair etc. cause individualistic variations in cancer risk and provide genetic severity to the disease pathology. In addition to genetic factors, the Human papilloma virus (HPV) also reported to have profound role in development of HNC. As the population of this region represents unique ethnicity, distinctive life style and food habits etc. which might play important role in the complex interplay of environmental and genetic factors that may be associated with high incidence of HNC in this region. Therefore, in the present study, we have tried to identify the susceptible genetic predisposition that could have played role in causation of head and neck cancers in the population of Northeastern region of India.

In this case-control study, a total of 205 newly diagnosed and histologically confirmed patients (Mean age 53.65 ± 12.15) and 210 matched healthy controls (Mean age 52.10 ± 12.32) (who were not diagnosed with cancer) and for the HPV

analysis of a total of 106 primary HNC tumor biopsy specimens were collected. These samples were analyzed for hr-HPV DNA (13 HPV types) using hybrid capture 2 (HC2) assay and genotyping was done by E6 nested multiplex PCR (NMPCR). The HPV cases were confirmed by immunohistochemistry (IHC) analysis of p16 gene were enrolled from the Dr. Bhubaneswar Borooah Cancer Institute (BBCI), Guwahati, India during 2011-2015. The study was approved by the Institutional Ethical Committee of Dr. Bhubaneswar Borooah Cancer Institute (BBCI), Guwahati, India. All the genotyping of xenobiotic, alcohol metabolism and DNA repair genes were determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) or polymerase chain reaction-confronting two-pair primers (PCR-CTPP). Genetic polymorphism, HPV status and their association with occurrence of HNC in presence of risk factors were analyzed by calculating odd ratios (ORs), 95% confidence intervals (95% CI) and corresponding P- values using SPSS software version 20.0 and Epi-info Version 6 software.

The CYP1A1 and CYP2E1 genes are involved in activation of major pro-carcinogens. We observed that association between CYP1A1 and CYP2E1 homozygous having 3.41 and 6.59 fold increased risk among betel nut chewers. Whereas CYP1A1 homozygous having 5.09 increased fold in tobacco chewers and CYP2E1 exhibited 10.23 fold risk in tobacco chewers. Among GSTs, GSTT1 null showed 1.73 fold increased risk in betel nut chewers, 2.86 fold in tobacco chewers and 2.39 fold in smokers. A notable finding of the study is the risk for HNC has been increased by the synergetic effect of betel nut, tobacco chewing and smoking habits in CYPE1 variants (OR^a, 10.43, CI 95% 2.74-39.66), when compared with betel nut and tobacco chewing alone.

We have also investigated the effect of genetic polymorphism of genes mainly involved in metabolism of polycyclic aromatic hydrocarbons (PAHs), N-nitrosamine, aromatic amines and ethanol such as EPHX1, NAT1 and NAT2 genes polymorphism in HNC. We found that the EPHX113 (CC) genotype has increased risk for HNC with exposure of betel nut (3.37 fold), tobacco (3.85 fold) and betel nut-tobacco (3.45 fold) and, EPHX139 (AG) genotype was found to have increased

risk with betel nut (3.42 fold), smoking (3.12 fold) and betel nut-tobacco-smoking, (5.22 fold). The TC and CC genotype of EPHX113 and AG genotype of EPHX139 increased the risk for HNC in betel nut alone and in combination with betel nut-tobacco chewing (both doses) cases. The NAT2 C481T genotypic variation (CT and TT) was found to be associated with HNC risk exposure with only betel nut. The NAT2 G590A (GA) genotype with betel nut chewing and (AA) genotype with smoking habit exhibited increased risk for HNC. However, NAT2 G857A genotype did not have any impact on HNC risk in the population of North-East India. All the doses of betel nut and tobacco with NAT2 C481T genotype and higher doses with NAT2 G590A showed strong impact on HNC risk.

The risk alleles of ADH2, ADH3 and ALDH2 genotypes plays important role in the development of several cancers however, their influence with alcohol drinking status in BN and tobacco associated HNC have not been investigated. Our case-control study demonstrated that higher consumption of alcohol increased the risk for HNC in the BN (4.40 fold) and BN–tobacco (4.40 fold) cases, but lower doses were found to be protective. The risk alleles of ADH2 and ADH3 have strong impact and in combination exhibited synergistic effect on the HNC in the NE subjects. Our study further demonstrated that the ADH2*1/*2 allele increases the risk for HNC in BN (3.49 fold) and BN-tobacco ((3.49 fold) fold) case. The allele ALDH2*1/*2 or ALDH2*/*2 did not have impact on the risk for HNC, but interaction of this allele with BN, tobacco and alcohol drinking status exhibited 5.35 fold increased risk in BN-tobacco cases synergistically with higher intake of alcohol. 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.

DNA in most cells is regularly damaged by endogenous and exogenous mutagens. Unrepaired damage can result in apoptosis or may lead to unregulated cell growth and cancer**.** Therefore we studied the effect of these confounding factors on genetic damage. In this present study, 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 exhibited increased risk for HNC. However, MGMT

(Trp65Cys and Leu84Phe) gene did not show any risk of HNC in the population of North East India. The CT and TT genotype of XRCC1 was associated with the risk for HNC in cases with both doses (lower and higher) of betel nut alone and in combination with tobacco and alcohol. Similarly, the CA, AA genotype of XPD exhibited increases risk for HNC with both doses (lower and higher) of betel nut and in combination with tobacco and alcohol.

Human papilloma virus (HPV) associated 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. It was observed that the hr-HPV was present in 31.13% (n = 33) of the HNC cases as examined by nested multiplex PCR (NMPCR) and HC2 assay respectively. Among hr-HPV positive cases, only two prevalent genotypes, HPV- 16 (81.81%), 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. 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.

The finding of the present study clearly demonstrated that genetic polymorphism in xenobiotic metabolism, DNA repair, alcohol metabolism acts synergistically with the dietary habits and hr-HPV infection is found to be associated with alcohol and tobacco consumption in the process of HNC development in North-Eastern Region of India. The findings of the present study would help in assessing the severity of disease, or could act as prognostic or predisposition markers and help in the development of personalized effective therapeutic regimens based on pharmacogenomics principle for better management of disease severity and treatment.