Risk factors for Head and Neck Cancer

By

Devyani Dilip Gholap

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DECLARATION

I hereby declare that the investigation presented in the thesis has been carried out by me. The work is original and has not been submitted earlier as a whole or in part for a degree / diploma at this or any other Institution / University.

Devyani Dilip Gholap

List of Publications arising from the thesis

Journal

"Ecological Analysis to Study Association between Prevalence of Smokeless Tobacco Type and Head and Neck Cancer", **Gholap DD**, Chaturvedi P, Dikshit R., *Indian Journal of Medical and Pediatric Oncology*,**2018**,*39*,456-462

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SYNOPSIS

INTRODUCTION

In 2018, 705,781 cases were diagnosed with head and neck cancers (lip, oral cavity and pharynx) worldwide and there were 1.8 million people alive who have been diagnosed with head and neck cancer in previous 5 years. The South East Asian region has highest age standardized incidence compared to other regions [1].

According to Global Adult Tobacco survey (GATS), 20.6% of Indian adults aged ≥15 years reported current use of smokeless tobacco only and 8.7% of adults reported use of smoked tobacco only, whereas 34.6% of adults reported dual use of tobacco[2].Within India it has been observed that there is huge variation in the age standardized incidence rates of different sub-sites of head and neck cancers [3].

Majorly head and neck cancers are due to exposure to carcinogens primarily due to lifestyle behaviors such as tobacco smoking, tobacco chewing and alcohol drinking. Factors like dietary intake and socio-economic status are strongly associated with head and neck cancers. Tobacco chewing behavior is more peculiar to Indian sub-continent and South East Asia than other parts of the world. Cohort studies conducted in south India have found increased risk with *Betel quid* chewing for oral cavity cancer in both sexes; the risk was further increased with higher frequency and longer duration of chewing [4] [5]. Pooled case-control study analysis have also found strong and independent association between smoking and head and neck cancer sub-sites [6] [7]. The risk further increases with increased frequency and duration. The attributable fraction of smoking in head and neck cancer is observed to be lower in younger adults (<45 years) than older adults. [8]. Cigar smoking risk is found to be higher than ever pipe and cigarette smoking [7]. Cigar and *bidi* smoking has been found to be strongly associated with

Oropharynx, Hypopharynx than Oral cavity cancers in Indian population [9]–[11]. Tobacco chewing behavior is more peculiar to Indian sub-continent and South East Asia than other parts of the world. The use of *Betel quid* also known as *paan* is extremely widespread across India. *Paan* is a mixture of areca nut, catechu (areca catechu), slaked lime, with or without tobacco and additional spices, wrapped in a betel leaf. Increased risk with *Betel quid* chewing for oral cavity cancer has been observed in both sexes; the risk was further increased with higher frequency and longer duration of chewing [4], [5]. Meta-analysis and case-control studies have found *Betel quid* without tobacco or areca nut chewing to be independent risk factor for oral cavity and hypopharynx cancer [12]–[17]. Tobacco chewing has been to be risk factor for oropharyngeal cancer [11], [15], [18]. Alcohol drinking attributes 3.6% of all cancer related cases and roughly 3.5% of all cancer related deaths [19]. The population attributable risk (PAR) of alcohol alone in HNC is less than 1% and 44% by tobacco and alone and is higher in pharyngeal caners [20]. Alcohol consumption (at highest frequency) has found to be associated with head and neck cancer among never smokers; the association is limited to pharyngeal cancers [21]. Alcohol drinking and tobacco smoking have multiplicative joint interaction in association with head and neck [22]–[24]. Along with these lifestyle factors, lowest levels of household income and educational attainment is associated with more than 2 fold increased risk of HNC and it is not entirely explained by behavioral risk factor differences [25], [26].

Human papilloma virus (HPV) infection is now also recognized risk factor for head and neck cancer especially for oropharyngeal cancers [27]–[29]. However, in India there are inconclusive studies on the role of HPV in association of head and neck cancers. Diet rich in fruits and vegetables have found to be protective, whereas red meat has found to increase head and neck increase risk [30]–[32].

GAPS IN LITERATURE

India has high HNC burden inspite of numerous efforts by the government and various prevention policies like enforcement of the 'Cigarettes and Other Tobacco Products (Prohibition of Advertisement and Regulation of Trade and Commerce, Production, Supply and Distribution) Act, 2003 (COTPA)' and tobacco control initiatives like National Tobacco Control Programme [33]–[35]. This high burden is hypothesized to be attributable to primarily tobacco chewing and alcohol drinking. There are however limited studies in India show stratified risk of various tobacco products which are commonly chewed such as *Gutka, Mawa, Khaini* and *masheri.* There is huge heterogeneity in smokeless tobacco (SLT) consumption across India and very few studies have explored the role of each SLT in relation to HNC risk. The role of SLT use in OPX, HPX and LX development is still not clear. Similarly, very few studies have studied alcohol's role with regards to HNC risk. Tobacco smoking and alcohol drinking lifestyle habits commonly coincide with each other. There are few studies in India which have studied the synergistic (joint) association of both lifestyles on HNC risk. The prevalence of HPV is well known in developed countries. In India however, there are no properly designed prevalence study with highly standardized protocol for HPV detection. The true prevalence of HPV and its genotype distribution in head and neck cancers is still unknown.

The present thesis proposal is designed to understand more clearly the role of SLT, alcohol and the prevalence of HPV in head and neck cancers.

HYPOTHESIS

- **Primary hypothesis**: Lifestyle habits like tobacco chewing, tobacco smoking and alcohol drinking increase the risk of developing head and neck cancers and the risk varies with different behaviours and tobacco and alcohol products.

- **Secondary hypothesis**: There is a significant difference between HPV prevalence across head and neck sub-sites.

AIM

Primary aim: To study the role of lifestyle factors in association of different sub-sites of head and neck cancer.

Secondary aim: To study the prevalence of *Human papilloma virus* in different sub-sites of head and neck cancer.

PRIMARY AIM

To study the role of lifestyle factors in association of different sub-sites of head and neck cancer.

Study Population: A hospital based case-control study was conducted at Tata Memorial Hospital (TMH), Mumbai from the period of January 2016 to March 2018.

Criteria for enrolment of cases: The cases were head and neck cancer patients of primary sub-sites oral cavity (International Classification of Diseases-Oncology [ICD-O] code C00-C06), Oropharynx (ICD-O code C09-C10), Hypopharynx (ICD-O code C12-C13) and Larynx (ICD-O code C32) visiting head and neck outpatient department of Tata Memorial Hospital. Primary cases aged 20-69 with date of diagnosis not more than 6months from date of interview were enrolled in the study. All the cases were histologically confirmed. Pregnant females were excluded from the study.

Criteria for enrolment of controls: All visitors with no history of cancer coming along with any site cancer patient aged 20-69 were included in the study. Visitor controls coming to various Disease Management Group (DMGs) have been enrolled. Not more than 20% controls have been enrolled from any of the DMGs, to avoid selection bias.

The study has been approved by TMH Institutional Review Board. Written informed consent was obtained from all participants before enrolling them in the study.

Data Collection: In-person interview of each case and control was conducted by trained interviewers using a pre-tested structured questionnaire covering demographic and socioeconomic variables, occupational history, personal and family medical history, tobacco and alcohol habits, and diet. Controls were frequency matched to cases on age and region of residence (South, North, East, West and Central India). For homogeneity in data collection social investigators were trained for questionnaire filling and data entry according to the instruction manual prepared for earlier case-control studies [36], [37]

Tumour tissue collection: $0.5cm^3$ size tumour tissue was collected for detection of HPV genotype from primary sub-sites- Oral cavity, Oropharynx, hypopharynx and larynx. The tissues were collected from minor and major operation theatres. The tissues were either biopsy or surgically resected specimens.

Definition of lifestyle exposures: A study participant was defined as a tobacco smoker if he has smoked one cigarette/bidi per week or $\geq$ 50 cigarettes/bidi over a period of six months, whichever was earlier. A packet of cigarettes was defined as 10 cigarettes and a packet of bidi was defined as 25 bidis respectively. Data was collected on the type of tobacco smoked cigarette, bidi or others (chillum, chutta, hookah, etc), the age at initiation and age of quitting, number of cigarette/bidi smoked in a day. A study participant was defined as a tobacco chewer if he/she has chewed at least once a week for six months or more. Data was collected on chewed tobacco preparation and its constituents such as lime, areca nut, betel leaf and catechu. Information was collected on consumption of commercial preparations of tobacco such as *Gutka* (dry mixture of crushed areca nut, tobacco, catechu, lime, aroma, flavoring and other additives), *Khaini* (tobacco

and slaked lime), *Mawa* (mixture of shaving of areca nut, scented tobacco, lime) and *masheri* (roasted, powdered preparation made by baking tobacco on a hot metal plate until it is uniformly black), *Lal dant manjan* and products without tobacco such as only areca nut chewing and *Paan masala.* The exposure measurement of tobacco use –smoke and smokeless consisted of past and current use. A study participant was defined as an alcohol drinker if he/she has consumed any type of alcoholic beverage at least once a week for six months or more. Data was collected on beer (brewed by fermenting malted barley), whisky (beverage made from distillation of fermented products of malted grains such wheat, maize, rye and oats), wine (beverage prepared by fermenting grape juice [white wine] or crushed grapes [red wine]), toddy (alcoholic beverage prepared from sap of various species of palm tree), country liquor (alcoholic beverage prepared by fermenting molasses of sugarcane) and other alcoholic drinks such as rum, brandy and vodka. Completed highest level of education was used as proxy variable indicator of socio-economic status. No education or illiterate was used as reference. Education was categorized into literate, < 5 years of schooling, 5-8 years of schooling, High school, college graduation and above.

Quality Assessment for Questionnaire Based Data

Monitoring of Daily Work: All forms were regularly checked for errors after conducting the interviews and after the data has been entered in the database. Weekly meetings were conducted to understand and resolve the problems of data collection. Training program was conducted every quarter to ensure the quality of interviews. The questionnaire was checked daily for completeness of information.

Quality Checks on Data Entry: Logical Checks were prepared to identify errors in the data entry. The data was entered twice and corrected for errors between 2 entries, if any, occurred while entering the data.

Reproducibility of Questionnaire: Abbreviated questionnaire was designed. This questionnaire contained constant (non changing in recent time) variables such as number of pregnancies, height, vegetarian /non-vegetarian status.

STATISTICAL ANALYSIS

Crude and adjusted Odds ratio and their 95% CI for developing head and neck cancer (HNC), oral cavity (OC), Oropharynx (OPX), hypopharynx (HPX) and larynx (LX) were calculated for tobacco smoking, tobacco chewing, alcohol drinking and socio-economic status. Unconditional logistic regression models were adjusted for potential confounders such as age (continuous variable), region of residence, gender, and socio-economic status. Association of tobacco smoking with HNC, OC, HPX and LX was evaluated by calculating pack years. For the calculation of pack-years, the amount of tobacco in grams was estimated as 1 per cigarette, 0.5 per bidi and 2 per cigar, cheroot and chutta. Alcohol drinking was measured by grams of ethanol, considering that one liter of ethanol weighs 798 g and that beer contains 5% ethanol in volume; wine 12%; liqueurs 30% and distilled spirits 41%. Joint association of tobacco smoking and alcohol were studied in relation to HNC, OC, OPX, HPX and LX. Models estimating risk of tobacco chewing were additionally adjusted for tobacco smoking (pack years) and alcohol drinking (ethanol gram years), while models assessing tobacco smoking were adjusted for tobacco chewing (never/ever) alcohol drinking (ethanol gram years). Similarly, models estimating risk of alcohol drinking were adjusted for tobacco smoking (pack years) and tobacco chewing (ever/never). Tests for linear trend across levels of exposure categories were performed on the continuous categorical variables entered as ordered, quantitative variables into the models. Test for heterogeneity to estimate differences in stratum specific odds ratio for HNC subsites (OC, OPX,HPX and LX) and types/products of tobacco smoking, tobacco chewing and alcohol

drinking was performed by using multinomial regression and Wald test testing the null hypothesis that the risk associated with the exposure was same across all sub-types. All analysis was performed using Stata statistical package version 15.0 [38].

Results: Questionnaire data was collected on 1320 head and neck cancer cases and 1924 controls. Oral cavity cases were 950, Oropharynx 166, Hypopharynx 117 and Larynx cases were 86. All the results were adjusted for the confounding variables unless mentioned otherwise.

Study participants who were ever smokers in lifetime had elevated risk of OR=2.0 (95%CI: 1.6-2.3) for head and neck cancer. The risk was higher for OPX (OR=5.68; 95%CI: 3.8-8.4) amongst all other subs-sites HNC. On further adjusting for additional lifestyle exposure variables such as tobacco chewing and alcohol drinking, the risk was further elevated for head and neck cancer OR=2.3(95%CI:1.9-2.8) and risk of OR=6.0(95%CI:4.0-9.0) was observed for Oropharynx which was still highest amongst all primary sub-sites of HNC. *Bidi* smoking had higher risk of all HNC sub-sites than cigarette smoking; HNC-OR=4.0(95%CI:3.1-5.3), OC-OR=2.0(95%CI:1.4-2.7), OPX-OR=11.6(95%CI:7.4-18.2), HPX-OR=5.5(95%CI:3.2-9.4), LX-OR=6.6(95%CI:3.8-11.5) after adjusting for confounders such as age, gender, region of residence, socio-economic status, tobacco chewing and alcohol drinking. With increasing smoking pack years, the risk increased linearly with highest risk for OPX OR=10.4(95%CI:6.2-17.5) at the highest quartile. Tobacco chewing showed elevated risk for all HNC sub-sites with highest risk for causing OC OR=8.7(95%CI: 7.1-10.7). *Gutka* chewing showed highest risk of OR=28.09(95%CI: 20.2-39) for causing OC amongst all other types chewing viz. *Khaini, Mawa, Mishri, Betel quid* with tobacco, tobacco quid, areca nut and products without tobacco. Tobacco chewed for >10 times a day showed highest risk for OC OR=14.8(95%CI: 9.7-22.5). Ever alcohol drinking has elevated risk for all HNC sub-sites, highest risk for OPX OR=1.8(95%CI:

1.2-2.8). Drinking country spirit has highest risk amongst all other types of alcohol viz. beer, whisky, toddy, vodka and rum. Risk for HNC cancer and its sub-sites increased with increase in duration of drinking years. The risk for cumulative drinking in ethanol gram years was highest for OPX OR=6.1(95%CI: 3.2-11.4) in highest quartile. Statistically significant effect modification was observed between tobacco smoking and alcohol drinking. Joint association with risk of OR=18.1(95%CI: 5.2-62.8) was observed for HNC and OR=8.2(95%CI: 1.9-35.0) was seen for OC in highest quartiles of drinking and smoking. Higher education was found to be protective for HNC and its all sub-sites compared to no education/illiterate. The association decreased linearly as years of education increased; most protective association was found for OPX OR=0.09(95%CI: 0.04-0.2).

SECONDARY AIM

To study the prevalence of *Human papilloma virus* in different sub-sites of head and neck cancer.

Tissue processing and DNA extraction: 175 tumour tissue samples were collected in tissue stabilization solution. The tissues were cut into small pieces and incubated overnight in tissue lysis buffer on a shaker incubator. Genomic DNA was extracted from lysed solution by using Qiagen DNAamp Tissue and Blood kit. Concentration of each DNA sample was determined by the optical density (OD) at 260 nm and the purification was evaluated by OD 260/280 ratio. The DNA aliquots were stored at -20ºC.

HPV Multiplex Polymerase Chain Reaction (PCR): The DNA samples were then subjected to multiplex PCR using biotinylated primers of 23 mucosal HPV genotypes[39]. DNAse/RNAse free water was used as negative control and Tris-EDTA buffer was used a blank for the PCR. Physical separation was maintained between PCR master-mix and final DNA addition steps to

avoid contamination. PCR tube strips were also used to avoid contamination. The total reaction volume was 25ul and following were the multiplex PCR conditions serially.

95°C	15mins	
94°C	30secs	45 cycles
63°C	3mins	
72°C	1.30 mins	
72°C	10min	
12°C	∞	

The PCR products were stored overnight at 4ºC and processed for Luminex assay next day.

Luminex assay: Luminex assay was performed to detect HPV genotypes. The PCR products were incubated with probe coupled polystyrene beads. The mixture is later heat denatured washed with wash buffer to remove unbound beads and stained with Streptavidin-Phycoerythrin conjugate to enable fluorescent detection of the bound beads specific to HPV genotype in Luminex analyzer.

Quality assessment: Intra and inter assay validation measures were used for quality control of the Luminex assay. Every Luminex 96-well plate was designed to run one sample in duplicate (intra- assay validation) and one sample was run from previous assay (inter- assay validation). Tris-EDTA buffer was used as blank and PCR negative control was run as Luminex negative control.

STATISTICAL ANALYSIS

Chi-squared test was used to determine joint association of HPV positivity and lifestyle exposure viz Smoking, chewing and alcohol drinking.

Results

The overall HPV prevalence in HNC was 39.43%; the prevalence sub-site wise was OC-36.27%; OPX-50%; HPX-50% and LX-26.32%. The most prevalent HPV genotype was HPV16 amongst all HNC sub-sites. HPV16 prevalence was highest in OPX and HPX (38.89%). HPV51 was most prevalent in HPX (11.11%) and HPV52 is most prevalent in LX (5.26%). However, HPV51 and HPV52 were borderline positives in all assays, on repeating they were borderline negatives. Thus, their prevalence should be treated with caution. HPV genotype co-infections were also observed. HPV co-infections of HPV16-HPV51, HPV16-HPV58 and HPV16-HPV58-HPV82 were observed. HPV16-58 co-infection was observed in all HNC sub-sites, most prevalent in OPX (11.11%). HPV16-HPV51 co-infection was observed in OC and OPX, most prevalent in OPX (2.78%). HPV16-HPV58-HPV82 co-infection was observed in LX only.

Lifestyle exposures smoking (ever/never), chewing (ever/never) and alcohol drinking (ever/never) were not found to be statistically significant with HPV positivity.

SUMMARY AND CONCLUSIONS

The strongest risk factors associated with head and neck cancer after adjusting for necessary confounding variables are as follows:

1. For every 500 smoking pack years increase the risk of HNC increases by 2.1 times, OPX by 2.4 times, HPX by 2.5 times, LX by 1.6 times. 1 smoking pack year equals to 1 pack of cigarette/bidi smoked daily for 1 year.

2. Amongst all chewed tobacco products *Gutka* chewing increases highest risk for Oral cavity cancer. For every 5 number/day increase in chewing any tobacco product the risk increases 1.2 times for HNC; 1.2 times for OC and 1.2 times for OPX.
3. Alcohol drinking increases risk for all HNC sub-sites. Drinking country spirit increases 2.5 times risk of OPX cancer. Increase in years of drinking duration increases risk of HNC and its sub-sites.
4. Higher education is negatively associated with HNC, OC, OPX, HPX and LX.
5. The overall HPV prevalence in head and neck cancers is 39.43% with HPV16 being the dominant genotype in all HNC sub-sites. HPV51, HPV52 and HPV82 are also prevalent in head and neck cancer either independently or co-infected with HPV16. However, further confirmation is necessary to conclude the finding.

   The current study concluded that tobacco and alcohol a substantially increase the risk of head and neck cancers in India. These lifestyle behaviors are 100% preventable owing to proper awareness and education of the masses. Prevention strategies should be aimed to reduce the lifestyle behavior. Reduction in tobacco smoking will be helpful to reduce the risk of other non-communicable diseases.

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List of Abbreviations

GATS	**Global Adult Tobacco survey**
HPV	***Human Papilloma virus***
HNC	**Head and Neck cancer**
OC	**Oral cavity**
OPX	**Oropharynx**
HPX	**Hypopharynx**
LX	**Larynx**
OSF	**Oral submucous fibrosis**
TMH	**Tata Memorial Hospital**
ICD-O	**International Classification of Diseases-Oncology**
OR	**Odds Ratio**
CI	**Confidence interval**
UI	**Uncertainty interval**
OD	**Optical density**
Tris-EDTA	**Tris-Ethylene diamine tetra acetic acid**
PCR	**Polymerase Chain Reaction**
TNM	**Tumour Node Metastasis**
IARC	**International Agency for Research on Cancer**
ASR	**Age standardized rate**
FCS	**Five year cumulative survival**
WHO	**World Health Organization**
PBCR	**Population based cancer registry**

SLT	**Smokeless tobacco**
AAR	**Age-adjusted incidence rate**
UADT	**Upper aero-digestive tract**
RR	**Relative risk**
INHANCE	**International Head and Neck Cancer Epidemiology**
AD	**Alcohol drinking**
SES	**Socioeconomic status**
ARCAGE	**Alcohol related cancers and genetic susceptibility in Europe**
TSNA	**Tobacco specific nitrosamines**
ETS	**Environmental tobacco smoke**
WCRF	**World Cancer Research Fund**
NRQ	**Non respondent questionnaire**
DNA	**Deoxyribonucleic acid**
RNA	**Ribonucleic acid**
RLB	**Reverse Line Blot assay**
HC2	**Hybrid Capture 2**
HR	**High risk**
MFI	**Median Fluorescence Intensity**
TS-MPG	**Type specific-multiplex genotyping**

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CHAPTER 1
INTRODUCTION

1.1 Biology of Head and neck cancer

1.2 Primary sub-sites of head and neck cancer

The structure of the upper aero-digestive cancers can be broken down into following primary sub-sites as the behavior of these sub-sites differs.

- Lip and Oral cavity
- Oropharynx
- Hypopharynx
- Larynx

Cancers of the oral cavity and Oropharynx are most common head and neck malignancies across the globe [40].

1.2.1 Anatomy of Oral cavity

The oral cavity (OC) is defined as the region from the vermillion of the lips anteriorly to the junction of the hard and soft palates posteriorly. Laterally, the oral cavity includes the anterior palatoglossal pillars and buccal mucosa. The sub-sites of the oral cavity are the lips, the buccal mucosa, the upper and lower alveolar ridges, the floor of the mouth, the anterior two-thirds of the tongue, and the hard palate [40]. The oral cavity is oval shaped and is separated into the oral vestibule and the oral cavity proper [41].

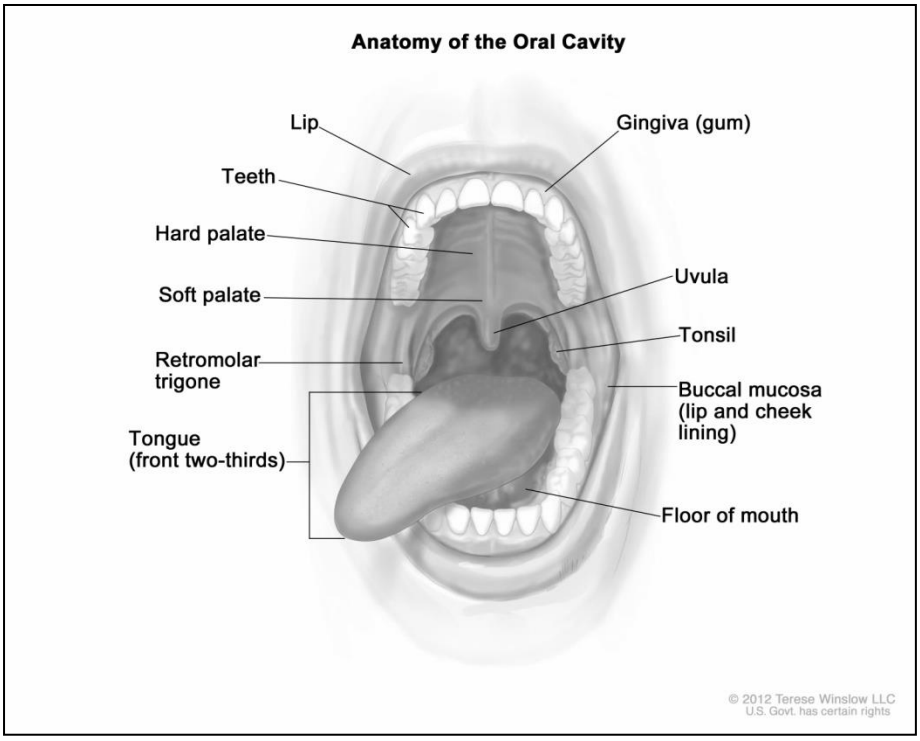

Figure 1. 1: Anatomy of Oral Cavity

The longer upper lip and shorter lower lip are connected to each other by the labial commissures at the corners of the mouth. The lips are separated from the cheeks by the nasolabial fold. The lip has an interior pale wet vermilion and an outer darker dry vermilion, separated from each other by the red line; there is also a white line on the outside that is the purely cutaneous part of the lip [41].

1.2.1.1 Cheeks (mucosa)

The cheeks are a musculo membranous structure and are limited superiorly and inferiorly by the upper and lower vestibules, anteriorly by the labial commissure, and posteriorly by the retromolar trigone and the intermaxillary commissure. The inner surface of each lip is connected in the middle line to the corresponding gum by a fold of mucous

membrane, the labial frenulum. The upper labial frenulum is larger than the lower labial frenulum. The retrocommissural region is situated between the labial commissure and the opening of Stensen's duct (the drainage duct of the parotid gland), located opposite the second upper molar. Stensen's duct runs through the buccinator muscle. A horizontal slightly elevated streak (called the linea alba or occlusal line) traverses this region level with the biting plane. The buccinator muscle forms the muscular framework of the cheek and is also a muscle of facial expression. It is covered by the buccal fat pad, which smoothes the cheek contour by filling in the depression and the anterior border of the masseter. The masseter muscle covers the buccinator. Other muscles also contribute to the formation of the cheek, such as the zygomaticus, risorius, and platysma [41]

1.2.1.2 Gums and alveolus

The gum (or gingiva) is a fibro epithelial mucosal tissue that surrounds the teeth and covers the alveolar jawbone. The alveolus is the tooth bearing area of the jaws. It is composed of a dense outer cortex (known as the cortical plate) and looser inner trabecular (or medullary) bone. The area of cortical bone that lines the dental socket (or alveolus) is called the lamina dura.

1.2.1.3 Retromolar trigone

The retromolar trigone is a small triangular shaped subsite of the oral cavity. It is the portion of mucosa that lies behind the third molar tooth, covering the anterior ramus of the mandible. The base of the triangle is posterior to the last inferior molar tooth; the apex is in continuity with the tuberosity of the maxilla behind the last upper molar tooth. The retromolar trigone is bounded laterally by the gingival buccal sulcus and medially by the anterior tonsillar pillar.

1.2.1.4 Hard Palate

The palate is the horseshoe shaped, domed roof of the oral cavity. It is divided into a hard portion and a soft portion. The hard palate belongs to the oral cavity and separates it from the nasal cavities. The soft palate belongs to the oropharynx and separates it from the nasopharynx. The hard palate is concave, and this concavity is occupied mostly by the tongue when it is at rest. The hard palate is subdivided into the primary and secondary palates. The primary palate is separated from the secondary palate by a small depression behind the central incisors termed the incisive fossa, where the incisive foramen opens. The anterior two thirds of the hard palate is formed by the incisive bone, or premaxilla, and the palatine processes of the maxilla. The horizontal plates of the palatine bone form the posterior third. The secondary palate presents a midline elevated suture line termed the median or palatine raphe. The hard palate also has transverse ridges (or rugae) on the anterior third that serve to retain the food bolus.

1.2.1.5 Floor of the mouth

The floor of mouth forms the inferior limit of the oral cavity. It is often compared to a quadrangular pyramid with a posterior base. Superficially, it is separated into 3 zones: the anterior floor of the mouth located anterior to the lingual frenulum and the 2 sublingual folds located between the lateral tongue and the mandibular gingiva. The sublingual papillae (also referred to as caruncles or folds) can be identified on both sides of the frenulum in the anterior part of the floor of mouth when the tip of the tongue is raised. The excretory duct of the sub-mandibular gland (Wharton's duct) runs in the floor of the mouth along the medial border of the sublingual gland to pierce the surface of the

mouth at the paramedian sublingual caruncle. The sublingual glands have multiple small ducts that drain directly into the floor of the mouth.

1.2.1.6 Tongue

The tongue is a mobile muscular organ that occupies the major part of the oral cavity and part of the oropharynx. Its main functions are pushing food into the oropharynx during swallowing and forming words during speaking, although it is also implicated in mastication, taste, and oral cleansing.

Because food is physically broken down in the oral cavity, this region is lined by a protective, non-keratinized, stratified squamous epithelium, which also lines the inner surface of the lips. The oral cavity proper is lined by a masticatory mucosa (gingiva and hard palate), a lining mucosa (lips,cheeks, alveolar mucosal surface, floor of the mouth, inferior surface of the tongue, soft palate), and a specialized mucosa (dorsal surface of the tongue)[41]

1.2.2 Anatomy of Oropharynx

The Oropharynx (OPX) is situated just posterior to the oral cavity and is an important and dynamic region for the functions of speech and swallowing. The oropharynx is bounded superiorly by a horizontal line through the superior surface of the soft palate and inferiorly by the superior border of the hyoid bone. Laterally, it is bounded by the tonsillar pillars and posteriorly by the posterior pharyngeal wall. Anteriorly, the Oropharynx ends at the most anterior extent of the soft palate, anterior tonsillar pillars, and the base of the tongue (that portion of the tongue posterior to the foramen cecum). The oropharynx is further subdivided into four subsites: the palatine tonsils and tonsillar

pillars, the base of the tongue, the soft palate, and the mid-posterior pharyngeal wall. A large amount of lymphoid tissue can be found in the oropharynx within Waldeyer's ring, which includes the tonsils bilaterally, the lingual tonsils, and the adenoid pad within the nasopharynx. The oropharynx is a region of the head and neck of great functional importance, both for maintaining a stable airway and for maintaining normal swallowing function.[40]

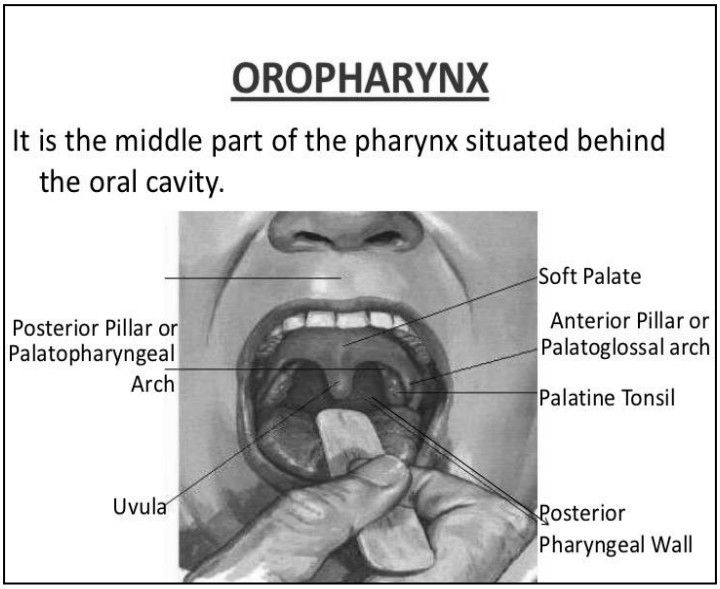

Figure 1. 2: Anatomy of Oropharynx

1.2.3 Anatomy of Hypopharynx

The hypopharynx (HPX) extends from the level of the hyoid bone above to that of the lower border of the cricoid cartilage below. For purposes of classification of the position and extent of carcinoma, the hypopharynx is divided into three areas:

- Pharyngo-oesophageal junction (postcricoid area): Extends from the level of the arytenoid cartilages and connecting folds to the inferior border of the cricoid cartilage.
- Pyriform sinus: Extends from the aryoepiglottic fold to the upper end of the oesophagus. It is bounded laterally by the thyroid cartilage and medially by the surface of the aryoepiglottic fold and the arytenoid and cricoid cartilages
- Posterior pharyngeal wall: Extends from the level of the vallecula to the level of the cricoarytenoid joints.

The hypopharynx is a mucosal lined tube related to muscle laterally and posteriorly and to laryngeal cartilages anteriorly. The muscle is the inferior constrictor. It arises anteriorly from the oblique line on the lateral side of the thyroid cartilage and the tendinous arch between the inferior tubercle of the oblique line and the cricoid cartilage. The lower part of the inferior constrictor is known as the cricopharyngeus muscle. The posterior attachment of the inferior constrictor on each side is to the pharyngeal raphe, a thin midline vertical tendon [42]

1.2.4 Anatomy of Larynx

The primary function of the larynx (LX) as a valve is to provide an airway for respiration and to protect the airway when swallowing. Its secondary function is to produce sound, which, in humans, we know as voice. The larynx is divided into three distinct anatomic divisions: the supraglottis, the glottis, and the subglottis. The supraglottis is composed of the epiglottis, the aryepiglottic folds, the arytenoid cartilages, and the false vocal cords. The glottis proper includes the superior and inferior surfaces of the true vocal folds and the anterior and posterior commissures. It extends from the lateral margin of the ventricle

to 1 cm inferiorly. The subglottis extends from the lower border of the glottis to the inferior border of the cricoids cartilage.[40]

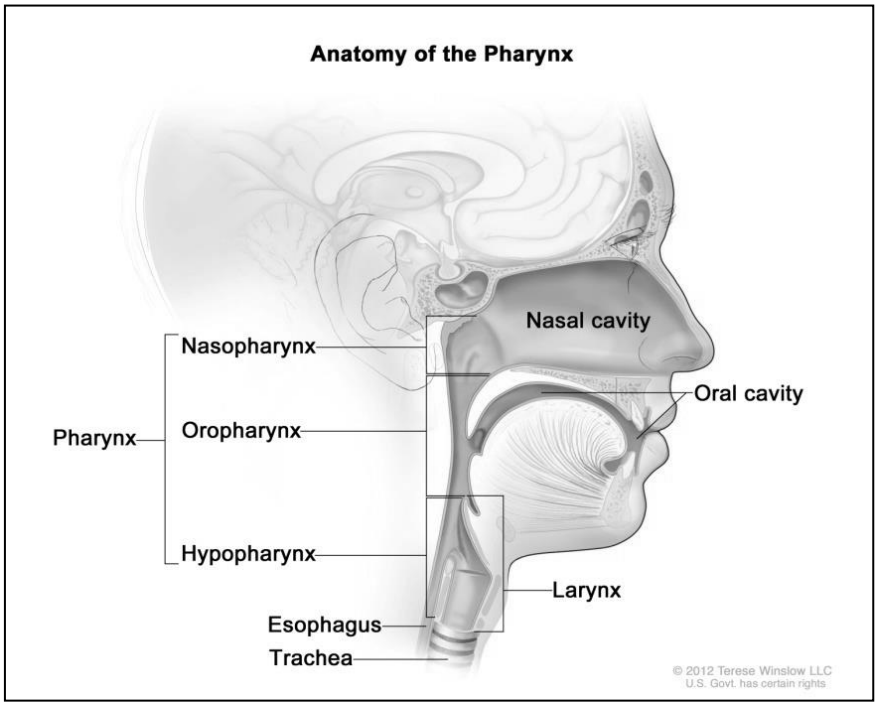

Figure 1. 3: Anatomy of the Pharynx

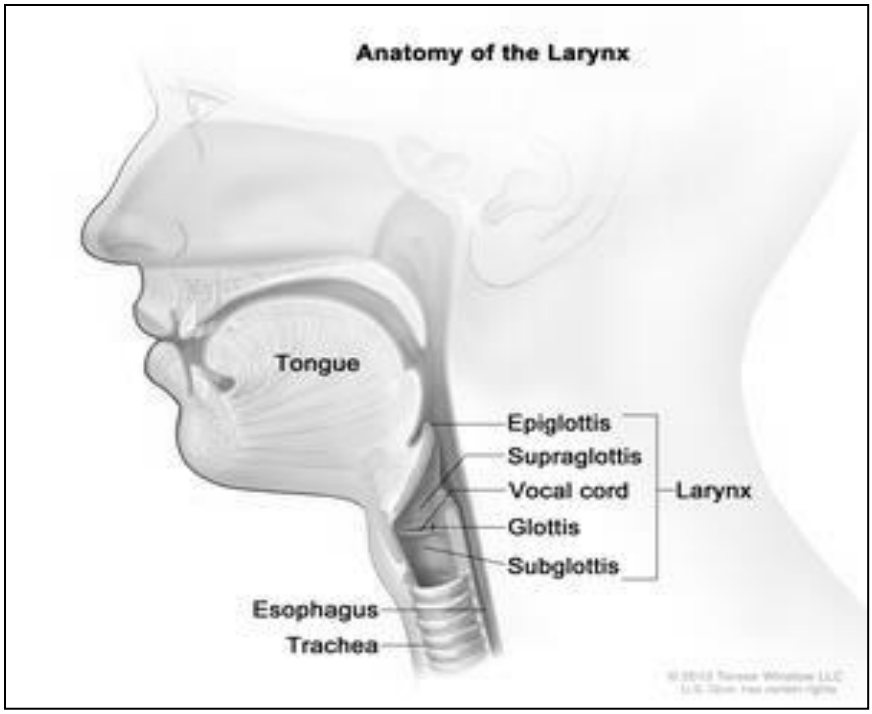

Figure 1. 4: Anatomy of the Larynx

1.2.5 Morphology of head and neck cancer

The majority of head and neck malignancies (90-95%) are squamous cell carcinomas. Squamous dysplasia includes changes like abnormal cellular organization, increased mitotic activity, and nuclear enlargement with pleomorphism. The microscopic appearance may vary as a function of tumor differentiation, but the prototypic head and neck squamous cell carcinoma (HNSCC) is moderately differentiated. The spindle-cell variant is characterized by the proliferation of non-cohesive spindle cells. Its microscopic appearance more closely resembles a sarcoma than a carcinoma. Verrucous carcinoma is seen clinically as an exophytic mass with a warty or papillary surface and has no potential to metastasize. The basaloid squamous variant is identified as a distinct subtype of HNSCC based on its striking basaloid morphology (e.g., solid lobules of cells with

peripheral pallisading, scant cytoplasm, and dark nuclei) and its highly aggressive behavior [43]. A study has shown the basaloid squamous cell carcinoma is a mixed group of *Human papilloma virus* (HPV) 16-positive and HPV-16-negative carcinomas [44].

1.2.6 Field cancerization

10-40% patients of HNSCC develop secondary tumours in regions of head and neck, lungs or esophagus. In 1953 Slaughter et al proposed an explanation for this multifocal tumour origin and termed it as 'field cancerization'. According to this concept, multiple cell groups independently undergo neoplastic transformation under the stress of regional carcinogenic activity. Slaughter and his group based this concept on the following observations: (a) tumor adjacent mucosa being molecularly 'abnormal' (b) multifocal areas of precancerous changes develop due to a prolonged and widespread exposure to carcinogens (c) oral cancer often consists of multiple independent lesions that sometimes coalesce and (d) formation of second primary tumors and recurrences can be explained by the presence of residual abnormal tissue after surgery [45]. It has been opined that a critical genetic alteration leads to transformation of the cell which provides a growth advantage amongst its neighboring cells. Collective observations have supported the view that these genetically altered cells populate the epithelium of mucosal tracts of upper respiratory tract and lack histopathologic evidence of dysplasia. The presence of these morphologically intact yet genetically altered cells can be explained by the phenomenon of field cancerization. [43].

1.2.7 Staging and tumour development

For each subsite of the head and neck, there is a different staging schema outlined by the American Joint Committee on Cancer, which follows the standard tumor, node, metastasis (TNM) staging system for malignancy. The nodes in the neck are divided into different levels and compartments for ease of classification. Level I is composed of the submental and submandibular triangles and is bounded posteriorly by the posterior belly of the digastric muscle. Level II contains the upper cervical nodes, extending from the skull base to the inferior border of the hyoid bone. Level III lymph nodes are found from the inferior border of the hyoid down to the inferior border of the cricoid cartilage. Level IV extends from the inferior border of the cricoid to the superior border of the clavicle. The third component of the assessment of stage is whether or not there are distant metastases. A designation of M1 is given to those patients with distant metastases; regardless of their T and N stage. The lymphatic nodes most at risk for metastasis from oral cavity malignancy are Level I, II and III. The Oropharynx has a rich lymphatic drainage system to levels II to IV and also drains to the retropharyngeal nodes. In advanced larynx cases where nodal metastasis in highly likely nodes level II and IV are most frequently involved.

1.2.8 Natural history of head and neck squamous cell carcinoma

Squamous carcinoma of head and neck cancer follow a definite histologic evolution starting from normal tissue to hyperplastic changes, to dysplasia, to carcinoma in situ, and finally to the development of an invasive carcinoma. Majority of HNCs progress in a predictable fashion from small primary lesion to larger lesion, to lymph node metastasis

and distant metastasis. The factors which influence the natural history of HNCs can be broadly studied under three subheadings: Primary lesion, lymphatic spread and distant spread.

Primary lesion: It forms one of the components of classical TNM (tumour, node and metastasis) staging system. Most HNCs begin as a surface each anatomical sub-site has its own particular pattern of spread. These cancers originate from the mucosal surface and a tumour may spread along muscle or facial planes for a considerable distance from the palpable or visible lesion. A tumour may attach to the periostium in very early in the course of the disease but actual bone or cartilage invasion is a late event. This pattern of local spread is seen in OC, OPX and LX cancers. Perineural invasion is also an important pathway of tumour spread. It is a histological sign of the biological aggressiveness of the tumour and is independent of the size of the primary lesion. Local spread of HNC depends on grade of tumour differentiation. Poorly differentiated tumours tend to have rapid doubling time and metastasize earlier than more differentiated types.

Lymphatic spread: The status of regional lymph nodes is one of the most important parameters that determines prognosis in patients with HNC. The risk of lymph node metastasis may be predicted by the differentiation of the tumour, by the size and the depth of invasion of the primary tumour, and by the availability of the capillary lymphatics. The more the lymphatic spread, more the chances of recurrence. Lymph node involvement follows orderly progression. Well lateralized lesions spread to ipsilateral neck nodes.

Distant metastasis: Clonal selection of cells that overcome immunological and mechanical barriers and vascular invasion of tumour cells results into distant metastasis of the disease. Distant metastasis in the absence of nodal metastasis is very rare in HNC. Untreated HNC may shed tumour cells in the lymphatic system and produce distant metastasis while the lymph node is growing slowly to a size that can be detected. It is seen that patients with advanced nodal disease have a high incidence of distant metastasis particularly in the presence of jugular vein invasion or extensive soft tissue disease in the neck[46].

1.3 Descriptive Epidemiology

Head and neck cancers (HNC) continue to remain a significant public health burden worldwide, causing significant mortality and morbidity despite advances in clinical knowledge which allow their early diagnosis and treatment. A study published by Gupta B et. al in 2016 concluded that the HNC burden is shifting towards less developed countries [47]

1.3.1 Burden of head and neck cancer sub-sites

Worldwide annually there are 263,000 new cases and 127,000 deaths from lip and oral cavity cancer (ICD-10 code: C00–08) and 135,000 new cases and 95,000 deaths from pharyngeal cancer (ICD-10 code: C09–10, C12–14) according to International agency for Research on Cancer (IARC). These cancers are among five most reported cancers in South East Asia [15]. According to Globocan 2018, lip and oral cavity cancers are second most common cancer in Indian males and third most common cancer in Indian females.

The common cancers in Indian males are lung and lip, oral cavity whereas in females they are cervix and breast[1].

1.3.2 Incidence of head and neck cancer sub-sites

Worldwide lip, oral cavity and pharyngeal cancers have been estimated to be responsible for 529,500 incident cancer cases (70.8% or 375,000 men and 29.2% or 154,400 women), accounting for 3.8% of all cancer cases as per 2012 figures. By 2035 the figure is predicted to rise by 62% to 856,000 cases because of the change in demographics. Among the head and neck cancer sub-sites, oral cavity has highest frequency (202,000 cases) followed by Oropharynx (100,500 cases) and hypopharynx (60,800 cases) and lip (23,700 cases) as per 2013 [48]. OPX incidence among men has significantly increased in economically developed countries like United States, Australia, Japan and Slovakia. The magnitude of increase in incidence was significantly in younger age groups (<60 years). The reason for this significant increase in OPX incidence was due to HPV infection resulting from unprotected sexual behavior [49].

In India, lip and oral cavity cancers are most common in Indian males. The number of incident lip and oral cavity cancer cases in India in 2016 was 113 000 (95% UI 106 000–118 000). Substantial reduction in AAR of lip and oral cavity (6·4%; 95% UI 0·4–18·6) was seen from 1990 to 2016 in a recent study published by India State-Level Disease Burden Initiative Cancer Collaborators. The AAR for lip and oral cavity cancer varied 5.1 times among both sexes combined across the states of India in 2016. The number of incident pharynx cancer cases in India in 2016 was 65 000 (95% UI 58 000–70 000), 70·2% of which were in males[50].

According to Globocan 2018 lip and oral cavity cancer is second most cancer in Indian population (Figure 1.6). The ASR of lip and oral cavity cancers is 9.1,OPX is 1.4, LX is 2.3 and HPX is 2.0 per 100,000 [51].

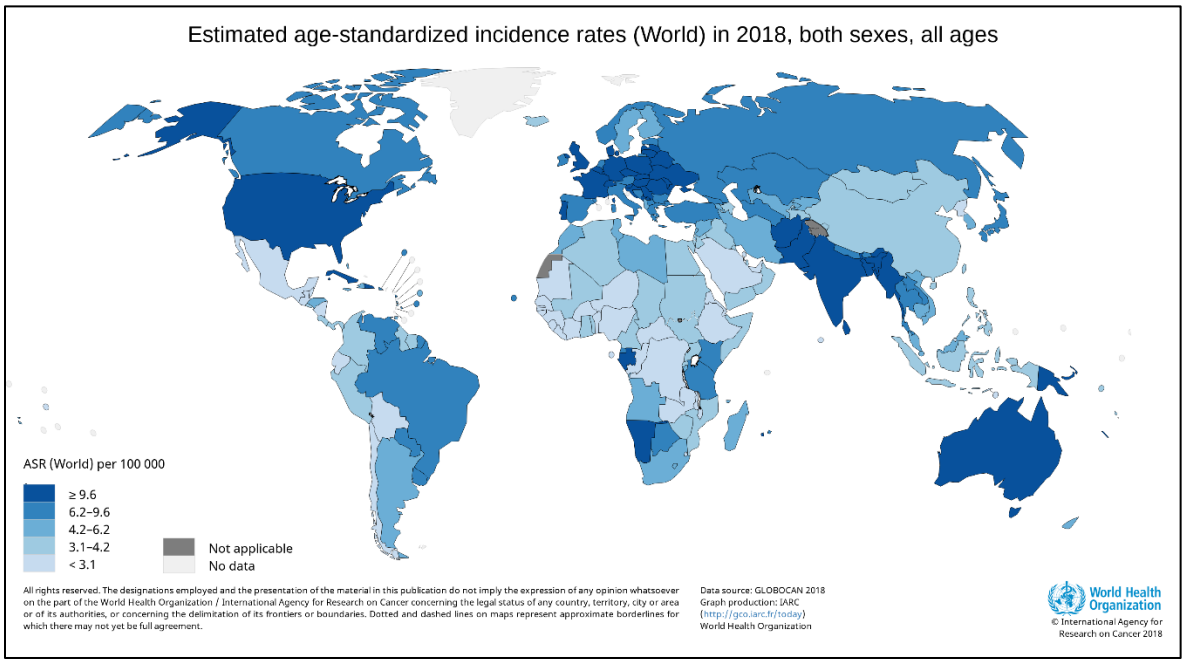

Figure 1. 5:Age standardized (world) incidence rate (per 100,000) of Lip, oral cavity, Oropharynx, Hypopharynx and Larynx cancer (all ages) of both sexes

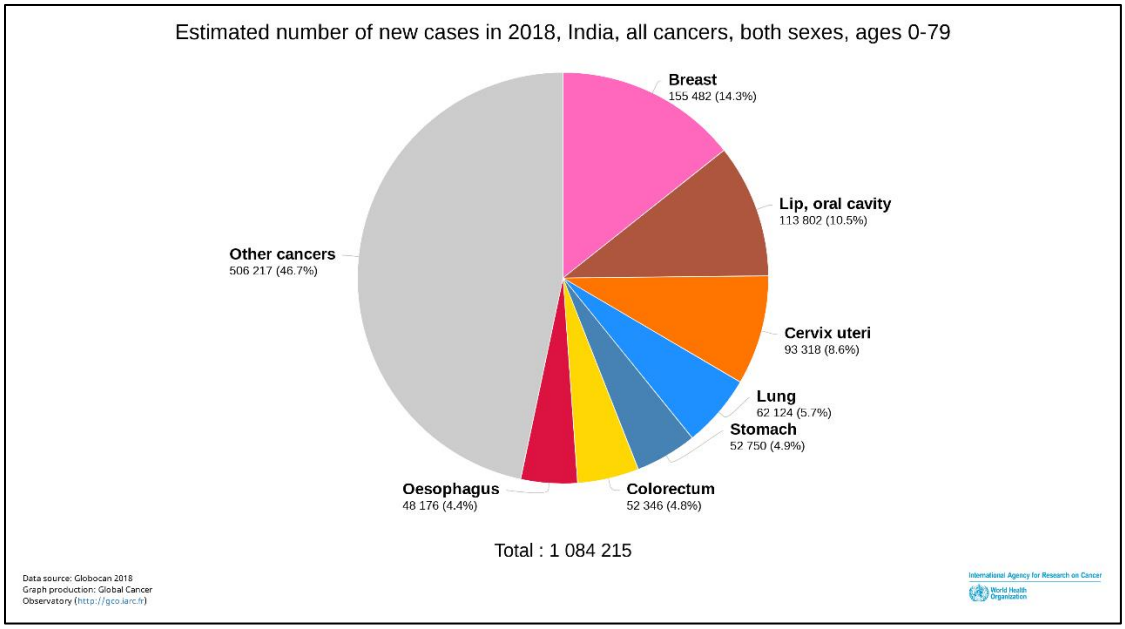

Figure 1. 6: Estimated number of new cancer cases in India in 2018

1.3.3 Survival of head and neck cancer

The overall 5-year survival for oral or oropharyngeal cancer is 65%; 33% for hypopharynx cancer and 61% for laryngeal cancers in the United States. Survival rates for oral and oropharyngeal cancers vary on factors such as original location of the tumour, HPV status of the individual, stage and extent of the disease. The survival rates are higher for patients with HPV infection [52]–[54]. Five-year cumulative survival (FCS) of tongue and mouth cancer was 67.5% and 60.4% respectively treated with surgery and radiation together in India (data extracted from hospital based cancer registries). FCS was 40% for oropharyngeal and hypopharyngeal cancers treated with chemotherapy [55]. A population based study which compared survival rates of developing and developed countries found 5-year survival of 60.2% for localized, 23.8% for regional and 3.3% for metastasized oral cavity disease in developing countries[56]. In

countries with racial profiling, differences in the outcome of head and neck cancer disease can be attributed to factors such as race, socio-economic status, access to health care and tumour stage [57], [58].

The level of development of health services and their efficiency to provide early diagnosis, treatment, and clinical follow-up care have a significant effect on HNC cancer survival. There is large variation in survival within populations in India which reflect differences in cancer related health services[56].

1.3.4 Mortality of head and neck cancer sub-sites

Worldwide, according to World Health Organization (WHO) in 2008, the age specific rate (ASR) due to lip and oral cavity cancers was 127,000; 95000 due to pharyngeal cancers and 82000 due to laryngeal cancers in both sexes [59]. Head and neck cancer mortality in India is at least half of incidence due to late presentation of patients for treatment [60]. Mortality due to oropharyngeal and laryngeal cancer is significantly higher in African American men which may be due to low prevalence of HPV positivity [61]. According to Globocan 2018, the age standardized mortality rate for lip and oral cavity is 7.0, OPX is 1.2, HPX is 0.69 and LX is 1.4 [62].

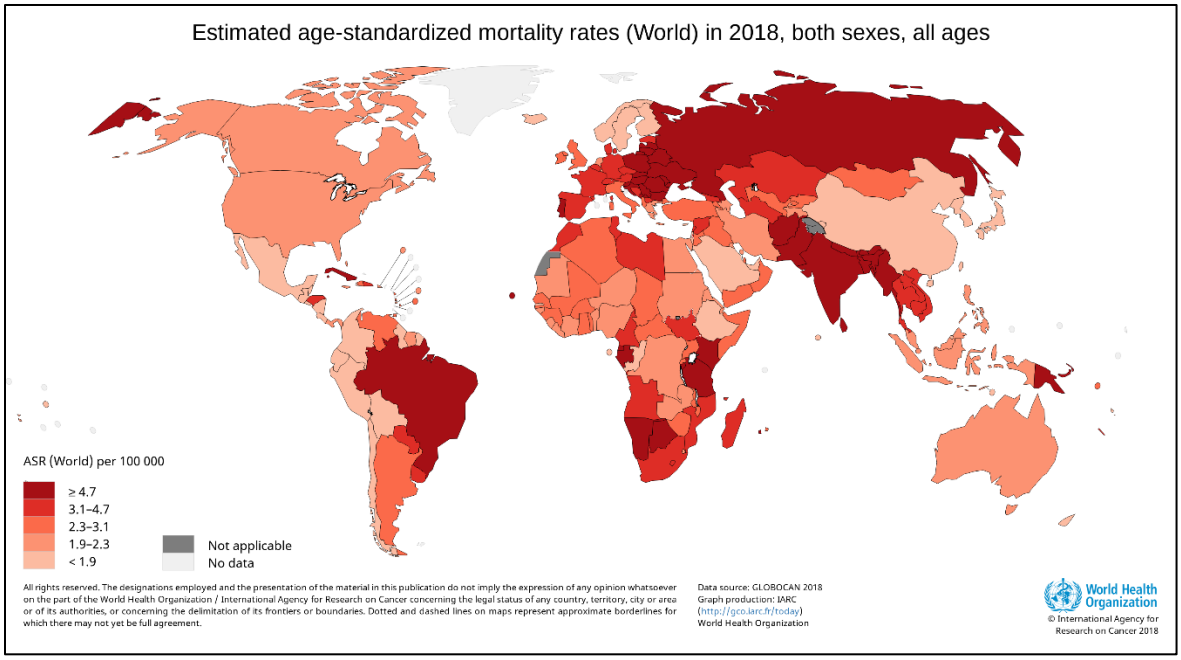

Figure 1. 7: Age standardized (world) mortality rate (per 100,000) of lip, Oral cavity, Oropharynx, Hypopharynx and Larynx cancer (all ages) of both sexes.

1.3.5 Differences in Incidence rates among Indian cancer registries

There are few studies in India reporting the difference in incidence of tongue, mouth, Oropharynx and hypopharynx cancers [63],[64]. These differences might be due to difference in the smokeless tobacco consumption (SLT) across regions in India. Table 1.1 and Table 1.2 shows AAR of all 29 population-based cancer registries (PBCR) and types of smokeless tobacco use (SLT) prevalence in Indian states in both sexes.

Table 1. 1:State-wise prevalence of smokeless products chewed and Age-adjusted incidence rates (AAR) corresponding to number of incident cases of HNC sub-sites of males

State	Prevalence of different smokeless products (%)					Age-adjusted incidence rate (AAR) of Head and neck cancer sub-sites			
	BQ+T[1]	*Khaini*	*Gutka*	Oral Tobacco[2]	Other smokeless products[3]	Tongue (AAR/no. of cases)	Mouth (AAR/no. of cases)	OPX (AAR/no. of cases)	HPX (AAR/no. of cases)
Nagaland	27.2	34.3	12	1.2	11.5	3.45/20	5.38/34	4.1/11	15.16/48
Tripura	27	9.1	2.5	0.5	3.9	4.16/198	4.45/217	1.43/67	4.43/207
Arunachal Pradesh	18.2	23.1	20.5	2.5	24.9	2.59/17	1.80/18	1.02/3	4.42/27
Assam	17.8	25.7	10.4	1.5	10.3	5.83/305	7.94/415	2.13/106	13.2/676
Karnataka	10.4	1.8	10.5	0.9	0.4	4.3/162	3.92/148	0.8/28	3.28/115
Sikkim	10.2	17.9	5.9	1.5	5	1.78/14	4.33/33	0.35/3	1.83/13
West Bengal	10	13.4	7.2	1.7	2.3	5.39/152	6.78/191	0.32/9	1.97/53
Madhya Pradesh	9.7	19.7	26.7	2.1	7.1	8.43/156	14.27/263	0.92/15	4.72/75
Kerala	8.7	3	1.6	1.3	2	5.8/625	6.56/700	2.02/215	1.95/204
Meghalaya	5.5	7.5	1.1	0.1	5.4	9.23/151	7.93/118	1.8/29	15.12/238
Gujarat	4.9	9.6	21.7	3.4	4.4	10.4/627	18.11/1113	0.54/29	3.51/180
Maharashtra	4.5	22.8	13.4	4.2	2.2	4.38/967	7.77/1736	0.37/106	1.48/380
Tamil Nadu	2.8	1.1	1.2	3.4	1.5	7.38/380	8.54/436	1.83/86	3.5/162
Mizoram	2.4	15.1	2.1	0.8	18.8	3.64/49	2.95/38	0.89/12	10.16/129
Delhi	2.2	5	13.2	0.5	0.7	9.33/659	9.46/703	1.77/112	2.29/136
Punjab	0.9	6.9	4.9	0.4	0	3.48/185	2.93/168	0.51/23	0.87/52
Chandigarh	0.5	5	3.2	0	0.3	4.3/23	4.2/25	0.5/5	2.3/3

Footnote: [1]*Betel quid* (BQ) with (+) tobacco (T); [2]snuff, *gul*,*gudhakhu* ,*Mishri*; [3]*Paan masala*,*Betel quid* without tobacco and nasal use of snuff.

The prevalence of chewing BQ + T in males is highest in Nagaland state (27.2%) followed by Arunachal Pradesh and Assam. The prevalence of chewing *Khaini* in males is highest in Nagaland (34.3%) followed by Assam and Arunachal Pradesh. The prevalence of chewing *Gutka* in males is highest in Madhya Pradesh (26.7%) followed by Gujarat and Arunachal Pradesh. The prevalence of chewing/applying oral tobacco products in males is highest in Maharashtra (4.2%) followed by Gujarat and Tamil Nadu.

The prevalence of chewing other smokeless products such as *Paan masala* and *Betel quid* without tobacco, in males is highest in Arunachal Pradesh (24.9%) followed by Mizoram and Nagaland.

Similarly, AAR of tongue cancer is highest in Gujarat followed by Delhi and Meghalaya. AAR of mouth cancer is highest in Gujarat followed by Madhya Pradesh and Delhi. AAR of Oropharynx cancer is highest in Nagaland followed by Assam and Kerala. AAR of Hypopharynx cancer is highest in Nagaland followed by Meghalaya and Assam.

Table 1. 2:State-wise prevalence of smokeless products chewed and Age-adjusted incidence rates (AAR) corresponding to number of incident cases of HNC sub-sites of females

State	Prevalence of different smokeless products (%)					Age-adjusted incidence rate (AAR) of Head and neck cancer sub-sites			
	BQ+T[1]	*Khaini*	*Gutka*	Oral Tobacco[2]	Other smokeless products[3]	Tongue (AAR/no. of cases)	Mouth (AAR/no. of cases)	OPX (AAR/no. of cases)	HPX (AAR/no. of cases)
Nagaland	22.6	17.4	7.4	0.5	15.3	1.52/10	1.94/12	0/0	6.81/7
Tripura	38.9	2.4	1.8	0.4	1.7	1.21/55	2.79/132	0.2/9	0.41/19
Arunachal Pradesh	10.3	13	11.4	2.2	16.2	0.63/8	1.5/12	0.14/1	0.2/2
Assam	11.4	2.3	4.1	1.9	10.4	2.36/109	4.67/218	0.8/31	2.53/120
Karnataka	9.5	2.9	1.1	2.9	3.2	1.17/42	5.38/179	0.16/5	0.78/29
Sikkim	4.2	10.9	6.8	0.2	7.5	0.48/4	2.52/17	0.15/1	0.39/2
West Bengal	8.5	4.2	1.7	7.1	1.8	2.36/60	3.01/77	0.16/4	0.42/10
Madhya Pradesh	3.7	8	6.4	7	4.7	3.66/58	5.51/85	0.07/1	0.34/6
Kerala	6.6	1.5	2.1	1.8	2.2	2.26/285	3.32/425	0.12/17	0.17/23
Meghalaya	23.4	4.2	1.4	2.6	8	2.22/36	7.71/125	0.26/4	2.24/37
Gujarat	1.9	0.3	0.1	0.7	0.5	3.39/188	3.63/197	0.13/6	0.9/52
Maharashtra	3.7	4.9	2.5	12.2	1.6	1.88/374	2.9/644	0.1/26	0.55/117
Tamil Nadu	6.6	0	0.3	0.3	1.7	2.03/101	3.99/190	0.24/11	1.52/78
Mizoram	11.7	34.3	6.2	5.6	18.1	0.84/10	1.66/21	0/0	1.15/14
Delhi	0.5	0.8	1.9	0	0.1	2.98/193	3.26/213	0.19/13	0.48/29
Punjab	0	0.1	0.1	0	0	0.85/49	0.87/48	0.21/4	0.3/17
Chandigarh	0.5	0.1	0.5	0.2	0.1	1.8/9	0.4/4	0/0	0.4/2

Footnote: [1]*Betel quid* (BQ) with (+) tobacco (T); [2]snuff, *gul*,*gudhakhu* ,*Mishri*; [3]*Paan masala*, *Betel quid* without tobacco and nasal use of snuff.

The prevalence of chewing BQ + T in females is highest in Tripura state (38.9%) followed by Meghalaya and Nagaland. The prevalence of chewing *Khaini* in females is highest in Mizoram (34.3%) followed by Nagaland and Sikkim. The prevalence of chewing *Gutka* in females is highest in Arunachal Pradesh (11.4%) followed by Nagaland and Sikkim. The prevalence of chewing/applying oral tobacco products in females is highest in Maharashtra (12.2%) followed by West Bengal and Mizoram. The prevalence of chewing other smokeless products, such as *Paan masala* and *Betel quid* without tobacco, in females is highest in Mizoram (18.1%) followed by Arunachal Pradesh and Nagaland.

Similarly, AAR of tongue cancer is highest in Madhya Pradesh followed by Gujarat and Delhi. AAR of mouth cancer is highest in Meghalaya followed by Madhya Pradesh and Karnataka. AAR of Oropharynx cancer is highest in Assam followed by Tamil Nadu and Punjab. AAR of Hypopharynx cancer is highest in Nagaland

1.4 Etiology

Majorly head and neck cancers are due to exposure to carcinogens primarily due to lifestyle behaviors such as tobacco smoking, tobacco chewing and alcohol drinking. Factors like dietary intake and socio-economic status also play a role in causation of HNC's. Every possible etiological factor in association with HNC is discussed further in detail.

1.4.1 Lifestyle exposures

1.4.1.1 Tobacco chewing

Smokeless tobacco is consumed without burning the product and can be used orally or nasally. Oral smokeless tobacco products are placed in the mouth, cheek or lip and sucked (dipped) or chewed. Tobacco pastes or powders are used in a similar manner and applied to the gums or teeth. Fine tobacco mixtures are usually inhaled and absorbed in the nasal passages [65]. The very first documented World Health Organization (WHO) report on epidemiology of oral and pharyngeal cancer in South east Asia with special emphasis given to tobacco chewing dates to 1966 by Takeshi Hirayama. He conducted retrospective case control studies and confirmed the association of tobacco chewing with oral cavity cancer. He also tried studying the 'dose response' relationship between chewing tobacco and oral cavity cancer and found that risk increased with increase in frequency of chewing

[66]. Tobacco chewing behavior is more peculiar to Indian sub-continent and South East Asia than other parts of the world. A relative risk of 15.07 with chewing >10 tobacco quids per day was observed by Sankaranaraynan et. al by conducting a matched case control study in Kerala [67]. The use of *Betel quid* also known as *paan* is extremely widespread across India. *Paan* is a mixture of areca nut, catechu (areca catechu), slaked lime, with or without tobacco and additional spices, wrapped in a betel leaf.

Table 1. 3: Classification of smokeless tobacco products by their use. Adapted from reference [65].

<table>
<tr><th colspan="3">Oral use</th><th>Nasal use (snuffing)</th></tr>
<tr><td>Chewing</td><td>Sucking</td><td>Other uses</td><td rowspan="2">Dry snuff</td></tr>
<tr><td>Betel quid</td><td>Dry snuff</td><td>Gudakhu</td></tr>
<tr><td>Gutka</td><td>Gutka</td><td>Gul</td><td rowspan="12">Liquid snuff</td></tr>
<tr><td>Khaini</td><td>Khaini</td><td>Mishri</td></tr>
<tr><td>Khiwam</td><td>Mishri</td><td>Red tooth powder</td></tr>
<tr><td>Loose-leaf</td><td>Maras</td><td rowspan="6">Tuibar</td></tr>
<tr><td>Mawa</td><td>Naswar</td></tr>
<tr><td>Plug</td><td>Snus</td></tr>
<tr><td>Tobacco chewing</td><td rowspan="3">Toombak</td></tr>
<tr><td>Gum</td></tr>
<tr><td>Zarda</td></tr>
</table>

Table 1. 4: Description of commonly used SLT products used in India. Adapted from reference [68], [69]

SLT products used commonly in India	Description
Khaini	Sun-dried tobacco and slaked lime are commonly used in the states of Gujarat and Maharashtra.
Zarda	Mixture of tobacco, lime, spices, and occasionally, silver flakes are added to *paan* and chewed together
Betel quid or paan	Contains betel leaf (*Piper betel*), areca nut, catechu, slaked lime, and tobacco. Spices and flavoring agents may also be added.
Kharra	Combination of tobacco, areca nut, lime, and catechu that is chewed in some parts of Maharashtra.
Gutka	Contains tobacco and is a ready to use packet product. It contains areca nut, slaked lime, catechu, and tobacco as well as flavoring agents and sweeteners that are added to improve taste. It is very popular in India due to its attractive marketing and packaging.
Mawa	Mixture of thin shavings of areca nut, tobacco, and slaked lime is widely preferred in Gujarat state.
Mainpuri tobacco	Tobacco preparation named after the *Mainpuri* district of Uttar Pradesh contains tobacco, slaked lime, areca nut, camphor, and cloves.
Gul or Gudhakhu	Dentifrice paste prepared from powdered tobacco and molasses. It is applied to the gums and teeth with a finger
Mishri/Masheri	It is made at home by roasting tobacco flakes on a hot griddle until it turns brown or black. It is applied to gums and teeth and retained in the mouth for variable time period.
Bajjar(dry snuff)	Tobacco product used mainly by women for cleaning teeth and gums.
Lal dantmanjan	Commercially available tooth powder.
Tuibar	Contains water through which tobacco smoke is passed. The water containing chemicals present in tobacco smoke is used for sipping or gargling in the northeastern states of India.

Other chewing tobacco products commonly used in India include *Khaini* (a mixture of tobacco and slaked lime), *Mawa* (tobacco, areca nut and slaked lime), *Gutkha* (tobacco, catechu, areca nut and slaked lime) and *Zarda* (tobacco and slaked lime). Snuffing of tobacco products (oral and nasal snuffing) also represents additional method to consume smokeless tobacco (SLT). A common snuffing product used is *Naswar* (mixture of tobacco and slaked lime). *Betel quid* chewing is well known to cause precancerous condition called as oral submucosal fibrosis and has also been identified as Group 1 human carcinogen by IARC. Carcinogenic compounds in SLT include polycyclic aromatic hydrocarbons, lactones, coumarin, ethyl carbamate, some volatile aldehydes, volatile N-nitrosamines, nitrosamino acids, tobacco specific N-nitrosamines, inorganic compounds, radioactive Polonium 210, and Uranium 235 and 238. N-Nitrosonornicotine (NNN), 4-(methylnitrosamino)-1 (3-pyridyl)-1 butanone (NNK), and N-nitrosamino acids are quantitatively the most prevalent strong carcinogens in SLT. The most abundant group of carcinogens found in SLT are tobacco specific N-nitrosamines (TSNA) and N-nitrosoamines [65]. NNK and NNN are classified as Group 1 human carcinogen by IARC. High levels of NNK and NNN were reported in *zarda* and *khaini* and TSNA level in *gutka* was higher than the permissible limits in food as per Indian regulatory laws [70].

Areca nut *(Supari)*, the seed from the areca palm *(Areca catechu)*, is a major ingredient of *gutka*, *Mainpuri*, *mawa*, *pan*, and some forms of *zarda.* Areca nut has been classified as a Group 1 human carcinogen by IARC. A study by Jacob et al observed definite dose-response relationship between areca nut chewing frequency and duration and oral submucous fibrosis development [71].

Cohort studies conducted in south India have found increased risk with *Betel quid* chewing for oral cavity cancer in both sexes; the risk was further increased with higher frequency and longer duration of chewing [4] [5]. Meta-analysis studies have also found tobacco chewing to be independent and strong risk factor for OC after adjusting for potential confounders [13]. Recently meta-analysis and case-control studies have found *Betel quid* without tobacco or areca nut chewing to be independent risk factor for OC and HPX [12]–[16]. IARC has classified '*Betel quid* without added tobacco' and areca nut as Group 1 human carcinogen. Pooled analysis from case-control studies have found ever use of nasal snuff to be associated with HNC particularly OC than chewing tobacco among never smokers [7].Studies have also shown tobacco chewing to be risk factor for oropharyngeal cancer [11], [15], [18]. There are limited studies on tobacco chewing and laryngeal cancers; however there are studies which have found tobacco chewing to be not an independent risk factor for LX [10], [72], [73].

To summarize smokeless tobacco has been found to be independent risk factor for oral cavity cancers and in few studies for pharyngeal cancers. There is significant dose-response relationship between tobacco chewing and oral cavity cancer, the risk increases with number of times tobacco chewed. There are limited studies on tobacco chewing and its association with pharyngeal cancers. *Betel quid* without tobacco and areca nut is associated with oral cavity, oropharynx and hypopharynx cancers. Their role in larynx cancer is still not known.

1.4.1.2 Tobacco smoking

Epidemiological evidence of tobacco smoking and cancer began to emerge in 1920's and in late 1950's causal relationship between tobacco smoking and lung cancer was

established [74]. Since then evidence of tobacco smoking and other parts of the respiratory tract like oral cavity, Oropharynx, hypopharynx and larynx began to accumulate. In 1985 under the aegis of IARC an international working group of experts found causal relationship between tobacco smoking and upper aero-digestive tract cancers (UADT)- OC,OPX ,HPX and LX [75]. Tobacco is smoked in the form of cigarette, cigar, *bidi*, *chutta* and kretek as described in the below Table 1.5.

Table 1. 5: Tobacco smoking products and their description. Adapted from reference [75].

Tobacco smoking product	Description
Cigarette	Any roll of tobacco wrapped in paper or other non-tobacco material; filter-tipped or untipped; approximately 8 mm in diameter, 70–120 mm in length.
Cigar	Any roll of tobacco wrapped in leaf tobacco or in any other substance containing tobacco Types: little cigars, small cigars (‘cigarillos’), regular cigars, premium cigars Some little cigars are filter tipped and are shaped like cigarettes. Regular cigars are up to 17 mm in diameter, 110–150 mm in length.
Bidi	Hand-rolled Indian cigarette; sun-dried *temburni* leaf rolled into a conical shape together with flaked tobacco and secured with a thread.
Chutta	Hand-rolled cigarette used for reverse smoking primarily by women in India.
Kretek	Small cigar containing tobacco (approximately 60%), cloves and cocoa. The burning blend gives a characteristic flavour and ‘honey’ taste to the smoke.

Tobacco smoke contains numerous carcinogens namely polynuclear aromatic hydrocarbons (PAHs), heterocyclic hydrocarbons, volatile hydrocarbons, nitrohydrocarbons, four aromatic amines, eight N-heterocyclic amines, N-nitroamines, two aldehydes, miscellaneous organic compounds, inorganic compounds and phenolic compounds. Compounds like 2-naphthylamine, 4-aminobiphenyl, benzene, vinyl

chloride, ethylene oxide, arsenic, beryllium, nickel compounds, chromium, cadmium and polonium-210 are classified as IARC Group 1 human carcinogens [75].

Meta-analysis study conducted by Gandini et. al analyzing tobacco smoking association with UADT in 2008 found highest relative risk (RR) of 6.98 for laryngeal cancer amongst other UADT cancers; followed by pharynx (RR=6.76) and OC (RR=3.43). The RR were for current smokers [76]. Netherlands cohort study (120,852 participants) found tobacco smoking to be strongly associated with OC, OPX, HPX and LX. The study found higher RR for OPX, HPX and LX over OC. They also found positive multiplicative interaction between smoking and alcohol drinking [77]. Pooled case-control study analysis have also found strong and independent association between smoking and HNC sub-sites [6] [7]. The risk further increases with increased frequency and duration. Pooled analysis from International Head and Neck Cancer Epidemiology (INHANCE) consortium found attributable fraction of smoking in HNC to be lower in younger adults (<45 years) than older adults. However they found positive association with pack years of smoking and HNC in young adults [8].Another pooled analysis by Wyss et al. from INHNACE consortium found ever cigar smoking risk to be higher than ever pipe and cigarette smoking [7].

In India most popularly smoked tobacco is in the form of *bidi.* A population based case control study conducted in central India by Dikshit et al concluded that *bidi* smoking is more harmful than cigarette smoking in development of OPX cancer [11]. Cigar and *bidi* smoking has been found to be strongly associated with OPX,HPX than OC in Indian population [9] [10].

1.4.1.3 Alcohol drinking

Epidemiological studies have reported alcohol drinking has substantial role in HNC development. There is convincing evidence that acetaldehyde, the first metabolite produced during alcohol degradation, is responsible for the carcinogenic effect of ethanol on the UADT owing to its multiple mutagenic effects on DNA. Alcohol related carcinogenesis may interact with other factors such as smoking, diet and co-morbidities, and depends on genetic susceptibility [78]. Alcohol drinking (AD) attributes 3.6% of all cancer related cases and roughly 3.5% of all cancer related deaths [19]. Meta-analysis studies have found positive association between AD and OC and OPC and the risk increases with increase in quantity [79] [80]. Pooled analysis study from INHANCE consortium found alcohol consumption (at highest frequency) to be associated with HNC development among never smokers; the association was limited to OPX,HPX and LX [21]. Netherlands cohort study found highest RR (RR=6.39) of alcohol consumption for OC than pharyngeal cancers [77]. Studies have found alcohol consumption to be strongly associated independently and jointly with smoking with LX cancer [81] [73] [82][83]. There are few and inconclusive studies on the risk of HNC and its sub-sites with respect to different alcoholic beverages. One of such pooled analysis suggested that risk of beer and liquor were comparable but found weaker association with moderate wine drinking. The authors however could not rule confounding from other lifestyle factors and diet [84]. Studies have concluded that alcohol drinking and tobacco smoking have multiplicative joint interaction in development of HNC [22]–[24]. In India, the types of alcohol consumed frequently are country liquor (locally brewed spirit containing 40% ethanol) and *toddy* (fermented sap from palm trees containing about 5% ethanol). Indian studies have also found alcohol drinking to be a strong risk factor for

HNC development [10], [67]. Karungappally cohort study conducted in south India found alcohol drinking risk to be more for LX than HPX [85].

1.4.1.4 Socio-economic factors

Besides other risk factors mentioned above, socioeconomic (SES) factors have been implicated to play and significant role in HNC development. SES factors compromise of education level, income, size of the household and number of people sharing a household. The best predictors for SES are variables such as education level and income. Most of the studies have taken in account these two variables as proxy to explore the association of SES in HNC development. Low SES has been strongly associated with HNC especially in men [25]. However these studies are usually limited to men with small sample sizes leading to imprecise estimates of true burden of SES [86] [87]. Pooled analysis from INHANCE consortium concluded that lowest levels of income and educational attainment were associated with more than 2-fold increased risk of HNC, which is not entirely explained by differences in the distributions of behavioral risk factors for these cancers and which varies across cancer sites, sexes, countries and country income inequality levels [26]. India has large class divide owing to the social and economic inequalities. However, there are no studies in India which study SES as a driving force for lifestyle behaviors (tobacco and alcohol use) in association with HNC. Mortality study conducted by Gajalakshmi et al among non-chewers and non-drinkers, education level was strong and independently associated (inversely) with mortality from mouth and pharyngeal cancers. However they determined cause of death by verbal autopsy [88].

SES is based on 3 inter-related dimensions, according to Weber: (i) class—incorporating ownership and the economic dimension, (ii) status—prestige or honor in the community

and (iii) power—political influence. The measurement of SES by household income, education attainment and occupation cover all these dimensions, but as these indicators are intertwined the mechanism for increased risk is thus complex [25].

1.4.1.5 Human papilloma virus (HPV) infection

HPV has been long known to important cause of anogenital cancer, in recent times it has been recognized as risk factor for subset of HNC. The proportion of HNC caused by HPV varies widely, largely because of the burden of tobacco associated disease in this population of tumours. Tobacco, alcohol, poor oral hygiene, and genetics remain important risk factors for HNC overall, but HPV is now recognized as one of the primary causes of OPX. In the USA, about 40–80% of OPX are caused by HPV, whereas in Europe the proportion varies from around 90% in Sweden to less than 20% in communities with the highest rates of tobacco use. Despite the recognized importance of HPV in many OPX cancers, the epidemiology of oral HPV infection is not well understood. Data suggest oral HPV prevalence is amplified with number of sexual partners and is more typical in men, in HIV-infected individuals, and in current tobacco users. Once the virus integrates its DNA genome within the host cell nucleus, it dysregulates expression of the oncoproteins E6 and E7. The E6 protein induces degradation of P53. The usual function of P53 is to arrest cells in G1 or induce apoptosis to allow host DNA to be repaired. E6 expressing cells are not capable of this P53-mediated response to DNA damage and, hence, are susceptible to genomic instability. The E7 protein binds and inactivates the retinoblastoma tumour suppressor gene product pRB, causing the cell to enter S-phase, leading to cell-cycle disruption, proliferation, and malignant transformation. Usually HPV positive OPX at presentation are at stage III or

IV [89]. There are over 100 known types of HPV, and they are classified into two types: a) low risk and b) high-risk. Various studies indicate that HPV 16 accounts for about 90% of all HPV-positive oropharyngeal cancer; whereas HPV 16, 18 and 33 combined account for about 98% of HPV-positive oropharyngeal cancers [90]. Meta-analysis studies have found HPV DNA presence to be highest in OPX in North America and Europe and in recent calendar time [27]. A systematic review analysis by Kreimer et al found overall HPV prevalence in HNC to be 25.9%, 35.6% in OPX; 23.5% in OC and 24.0% in LX. HPV16 genotype accounted for 86.7% of all OPX cancers [91]. Study conducted by Anantharaman et al on ARCAGE (Alcohol related cancers and genetic susceptibility in Europe) study samples found HPV16 E6 antibodies to be strongly associated with OPX. The results also suggested marginal role of HPV18 E6 and HPV6 role in LX. They also performed paired analysis (blood and tumour tissue of the same subject) and found 67% concordance between serological and genotyping results [29]. The HPV attributable fraction of HNC varies substantially between countries and is dependent on factors like tobacco smoking, alcohol, sexual lifestyle and oral hygiene. Even though India has high burden of HNC, the role of HPV in its development and prognosis is still unknown. In central India study conducted by Gheit et al found HPV DNA prevalence to be 13.7% in overall HNC, the highest was found for OPX (9.4%) [92].

1.4.2 Other factors

1.4.2.1 Environmental tobacco smoke

Environmental tobacco smoke (ETS), or involuntary smoking, comprises side stream smoke from the smoldering tobacco between puffs and exhaled mainstream smoke from the smoker. ETS has been recognized as a human carcinogen by a working group of the IARC. ETS exposure is assessed from partner, workplace and childhood exposure. ETS exposure has been found to be associated with HPX and LX after controlling for confounders (tobacco smoking, alcohol and education) [93]. Pooled analysis from INHANCE consortium observed no effect of ETS on HNC overall but found risk with long duration of ETS at work or home, the effect was stronger for OPX,HPX and LX [94]. Childhood passive smoking exposure is strongly associated with HNC specifically with OPX, HPX and LX [95].

1.4.2.2 Diet

Epidemiologic studies suggest that a high intake of fruits and vegetables is associated with decreased risk of cancers of the UADT. The evidence for a decrease in risk due to high fruits and vegetables intake was recently rated by an IARC-expert panel for cancers of the OPX and LX as ‘possible’. World Cancer Research Fund (WCRF) report on diet and cancer summarized that there is strong causal relationship between consumption non-starchy vegetables, fruits, and food containing carotenoids with decreased risk of HNC [96]. Poor diet characterized by low fruit and vegetable intake and high meat and fat consumption has been related to increased HNC risk. The inverse association of fruits and vegetables against HNC has been reported in many epidemiologic studies [97]–[100]. In a multicentric case control conducted by IARC concluded that

consumption of vegetables and fruits may modulate the carcinogenic effects of tobacco and alcohol [101]. The Carolina head and neck epidemiology study found that intake of vegetables, fruits and lean protein had protective effect on HNC. Intake of fried foods, sweets, high fast and processed meats increased the risk of LX [102]. Pooled analysis from INHANCE consortium (14,520 cases; 22,737 controls) found protective effect in highest quartile of vegetable and fruits intake for HNC. Intake of red meat and processed increased the risk of HNC. The protective effect of vegetables and fruits may be due to nutrients such as Vitamin C, Vitamin E, folate, fiber and flavonoids may alter the carcinogenic effects of smoking by reducing the smoke induced oxidative damage or inflammatory responses [30]. The mechanisms behind red meat and processed meat and cancer risks include iron over-storage or oxidative stress resulting from free radicals and carcinogens generated or added during meat preparation or preservation [96]. Intake of white meat (combination of poultry, fish, and shellfish) is observed to have inverse association with HNC risk [31]. Compared to red meat poultry and fish are lower in saturated fat and heme iron and may result in less exposure to free radicals or carcinogens generated during the processing of red meat or processed meat [31]. Case control studies have reported stronger associations between diet and HNC than cohort studies. While recall bias in case control studies might have resulted in stronger associations, on the other hand behavior change after recruitment in cohort studies might have diluted the associations. As pooled analysis studies have observed no heterogeneity between hospital based, population based case control studies and cohort studies, it gives out a uniform message that vegetable and fruit intake lower the risk whereas red meat intake increases the HNC risk [30]. There are limited studies in India with respect to diet

and HNC. Studies conducted in India gave similar conclusions as international studies, that frequent consumption of fruits and vegetables lower the risk of HNC [32] [103].

1.4.2.3 Occupation

Increased risk for HNC have been consistently found for number of occupations such as painters, specific categories of construction workers, metal workers, laborers, butchers and shoe and textile workers in European studies. Risk has also been found for occupational agents like sulfuric acid, asbestos and coal dust [104]. Analysis from ARCAGE case- control study found HNC risk to be higher for painters, bricklayers, workers involved in erection of roofs and frames, reinforced concreters, dockers and workers involved in construction of roads. The risks further increased for longer duration of employment. There was no association found among women [104]. Case control study conducted by Merletti et al found higher risk of OC and OPX for ever machine operators, plumbers, workers of building and textile industry after adjusting for all potential confounders [105]. A positive association was found for occupational agents like asbestos, solvents, formaldehyde with HPX and LX [106].

1.4.2.4 Oral hygiene

Oral hygiene is a practice of keeping the mouth and teeth clean to avoid dental problems, periodontal diseases and infections. Poor oral hygiene is known to contribute HNC risk although the causality and independency of the indicators is not known [107]. Oral hygiene indicators such has number of missing teeth, denture use, bleeding gums, tooth cleaning frequency, instrument used to clean teeth, dentist visiting frequency have been suspected to contribute to the etiology of head and neck cancers [107]. Studies have found that the oral fauna may vary in individuals with missing teeth which might

contribute to tobacco metabolism. Also, oral bacteria causing periodontal disease convert ethanol and dietary sugars to acetaldehyde which is a known carcinogenic metabolite of ethanol [108]. A study conducted in southern India by Balaram et.al found increased oral cancer risk associated with gum bleeding, use of finger to clean teeth and number of missing teeth after adjusting for smoking, tobacco chewing and alcohol drinking [9]. A multicentric case control study conducted by Guha et.al in central Europe and Latin America found poor oral hygiene to be strong risk factor for all subsites HNC especially OC. In central Europe brushing teeth less than once per day increased the risk of laryngeal cancer. Using fingers or stick to clean teeth and frequent gum bleeding increased the risk of oral cavity cancers, while never having dental check-up increased the risk of cancers of pharynx and larynx [109].

1.4.2.5 Genetic Susceptibility

A family history of cancer is another important risk factor for HNC, which implies that genetics contributes to HNC susceptibility. Given the biological plausibility of a theory that individual differences in biotransformation efficiency and detoxification of pro-carcinogens might be important HNSCC risk factors, most HNSCC susceptibility studies deal with the polymorphisms in genes encoding the enzymes involved in these reactions. Activated (pro) carcinogens present in tobacco (and its smoke) and alcohol, particularly polycyclic aromatic hydrocarbons and acetaldehyde, respectively, may react with the exposed mucosa of the upper aero-digestive tract and form DNA adducts. The latter can cause mutations in crucial genes involved in carcinogenesis, such as tumor suppressor genes or oncogenes, ultimately leading to cancer [110][111]. Considering only studies with sufficient statistical power, the most consistent significant associations

were obtained for genetic polymorphism in GST (*GSTM1null*), where the variant with absent enzyme function increases HNSCC risk. In a meta-analysis and pooled analysis, Hashibe et al found a borderline association with a modest increased risk of HNSCC for the *GSTT1* null, the *GSTP1* polymorphism (*ILE105VAL*), and the *CYP1A1* polymorphism (*CYP1A1 Val462*) with an absent or decreased enzyme function associated with an increased risk of HNSSC. Having all 3 of the above-mentioned GST polymorphisms led to a higher HNSCC risk in comparison with having only 1 [112][113]. A large genome-wide association (GWAS) study was conducted recently by INHANCE consortium. It found that 3 common genetic variants in alcohol metabolizing enzyme ADH were associated with an increased risk of HNSCC (rs1573496-ADH7, rs1229984-ADH1B, rs698-ADH1C). In the same study, the 12q24 variant (rs4767364) located in the region containing multiple genes including 1 encoding for another enzyme involved in alcohol metabolism, ALDH2 was associated with an increased risk of HNSCC[114]. A meta-analysis concluded that genetic polymorphisms in DNA repair pathway namely, *XRCC1* codon 194 (in Asians), *XPD* codon 156 (in Caucasians), and *XPD* Asp312Asn (in a mixed Asian and Caucasian population) may be associated with an increased risk for HNSCC [115]. INHANCE consortium found DNA repair-related genes *HEL308* and *FAM175A* to be associated with an increased risk for HNSCC[114]. Impaired apoptotic pathways have also found to increase HNSCC risk. Zhou e.t al. reported an association presence of rs3810294 and of rs2032809 polymorphisms in the promoter of the p53 upregulate modulator of the apoptosis (*PUMA*) gene and increased HNSCC risk. The risk modifying effect was much stronger for HPV16-positive patients with oropharyngeal carcinoma[116]. HPV infection causing HNSCC leads to integration

of viral DNA in the host genome, thus development of cancer depends on individual susceptibility. Susceptibility marker *TGF-β1* variants are twice as likely associated with HPV16 positive infection as patients with no variants[117]. MDM4 is a protein found to regulate p53 expression and thus possibly lead to carcinogenesis. *MDM4* polymorphisms have found to have modifying effect on HPV16 serology and risk of OPX, the effect is more prominent in never smokers and never drinkers[118]. Other genetic polymorphisms with potential impact of risk of HNSCC are polymorphisms in mitochondrial DNA (mtDNA), polymorphisms in bilirubin-related pathway, *E2F* transcription factors and epigenetic variations [113].

Table 1. 6: Risk factor summary for head and neck cancer

Risk factor	Direction of effect
Established risk factors	
Tobacco chewing	⇧
Tobacco smoking	⇧
Alcohol	⇧
HPV infection (for OPX)	⇧
Possible risk factors	
High intake of fruits and vegetables	⇩
High intake of red meat	⇧
High intake of processed good and high fats	⇧
High intake of white meat	⇩
Insufficient risk factors	
Occupation (manual worker and laborer)	⇧
Low socio-economic status	⇧
Environmental tobacco smoke	⇧
Poor oral hygiene	⇧
HPV infection (for OC and HPX)	⇧
Genetic susceptibility	⇧

⇧: Increase in risk; ⇩: Decrease in risk

Source: Textbook of Cancer Epidemiology, Second Edition, Hans Olav Adami, David Hunter, Dimitrios Trichopoulos

1.4.3 Gaps in Literature

India has high HNC burden inspite of numerous efforts by the government and various prevention policies like enforcement of the 'Cigarettes and Other Tobacco Products (Prohibition of Advertisement and Regulation of Trade and Commerce, Production, Supply and Distribution) Act, 2003 (COTPA)' and tobacco control initiatives like National Tobacco Control Programme [33]–[35]. This high burden is hypothesized to be attributable to primarily tobacco chewing and alcohol drinking. The population attributable risk was 66.1% for SLT in OC development and 71.6% for smoking in OPX development[11]. There are however limited studies in India show stratified risk of various tobacco products which are commonly chewed such as *Gutka, Mawa, Khaini* and *Mishri*. There is huge heterogeneity in SLT consumption across India and very few studies have explored the role of each SLT in relation to HNC risk. The role of SLT use in OPX, HPX and LX development is still not clear. Similarly, very few studies have studied alcohol's role with regards to HNC risk. Tobacco smoking and alcohol drinking lifestyle habits commonly coincide with each other. There are few studies in India which have studied the synergistic (joint) effect of both lifestyles on HNC risk. The prevalence of HPV is well known in developed countries. In India however, there are no properly designed prevalence study with highly standardized protocol for HPV detection. The true prevalence of HPV and its genotype distribution in head and neck cancers is still unknown.

The present thesis proposal is designed to understand more clearly the role of SLT, alcohol and the prevalence of HPV in head and neck cancers

Hypothesis

- **Primary hypothesis**: Lifestyle habits like tobacco chewing, tobacco smoking and alcohol drinking increase the risk of developing head and neck cancers and the risk varies with different behaviours and tobacco and alcohol products.
- **Secondary hypothesis**: There is a significant difference between HPV prevalence across head and neck sub-sites.

CHAPTER 2
LIFESTYLE AND HEAD AND NECK CANCER

2.1 Introduction

Following sections are described in detail:

- Methodology and quality measures for questionnaire based study.
- Lifestyle factors and HNC risk.
- Socio-economic status and HNC risk.

2.2 Study design

A hospital based case control study was conducted in Tata Memorial Hospital (TMH), Mumbai during the period of January 2016 to March 2018

2.2.1 Criteria for enrolment of cases

The cases were head and neck cancer patients of primary sub-sites oral cavity (International Classification of Diseases-Oncology [ICD-O3] code C00-C06), Oropharynx (ICD-O3 code C09-C10), Hypopharynx (ICD-O3 code C12-C13) and Larynx (ICD-O3 code C32) visiting head and neck outpatient department of Tata Memorial Hospital. Primary cases aged 20-69 with date of diagnosis not more than 6months from date of interview were enrolled in the study. All the cases were histologically confirmed. Pregnant females were excluded from the study.

2.2.2 Criteria for enrolment of controls

Visitors accompanying any cancer patient to the hospital aged between 20-69 years were included in the study. None of the cancer site patients with which visitors came along with, constituted more than 20% of enrolled controls. The maximum number of enrolled visitor control came along with gastrointestinal cancer patients while the minimum number of enrolled visitor controls came along with hematolymphoid tumours. The selected controls thus belonged to the

same study base from which cases were coming to TMH and the selection bias was kept at minimum. Pregnant females were not included in the study.

2.2.3 Matching

Controls were frequency matched to cases on age (± 10 years), region of residence at the time of enrolment and gender. For region matching, India was divided into five regions which are as follows:

1. North (Haryana, Uttar Pradesh, Himachal Pradesh, Delhi, Punjab, Uttarakhand, Rajasthan, Bihar, Chandigarh, Jammu & Kashmir)
2. South (Kerala, Tamil Nadu, Andhra Pradesh, Karnataka, Puducherry, Lakshadweep, Andaman & Nicobar Islands)
3. East (Arunachal Pradesh, Assam, Meghalaya, Manipur, Mizoram, Nagaland, Tripura, West Bengal, Orissa and Jharkhand)
4. West (Maharashtra, Goa, Gujarat, Dadra Nagar Haveli, Daman & Diu)
5. Central (Chhattisgarh and Madhya Pradesh).

The cases and controls were recruited simultaneously during the study period**.** The study has been approved by TMH Institutional Review Board. Written informed consent was obtained from all study participants before enrolling them in the study.

2.3 Data collection

2.3.1 Questionnaire data

The questionnaire consisted of demographic and socioeconomic status, reproductive history, time spent in household activities on a normal day, residential history, occupational history, personal and family medical history, diet, tobacco and alcohol habits. Tumour tissue clinical questionnaire was developed which consisted of histology, tumour staging, grade of tumour and treatment of cancer details.

2.3.2 Tumour tissue collection

Tumour tissue was collected wherever feasible. Surgical and biopsy specimens were collected in tissue stabilization reagent RNA later®. The tissues were collected at room temperature. The collected tissues were incubated at 4ºC overnight. After overnight incubation the tissue was removed from RNA later® and stored for long term period at -80ºC. The tissues were uniquely coded and barcode labeled for efficient storage and retrieval.

2.3.3 Non-respondent questionnaire

Non-respondent questionnaire (NRQ) was designed to estimate the response rate of the study and to check for non-response bias. The questionnaire was piloted and implemented with training to the interviewers. The questionnaire consisted of case details and reasons for non-participation in the study.

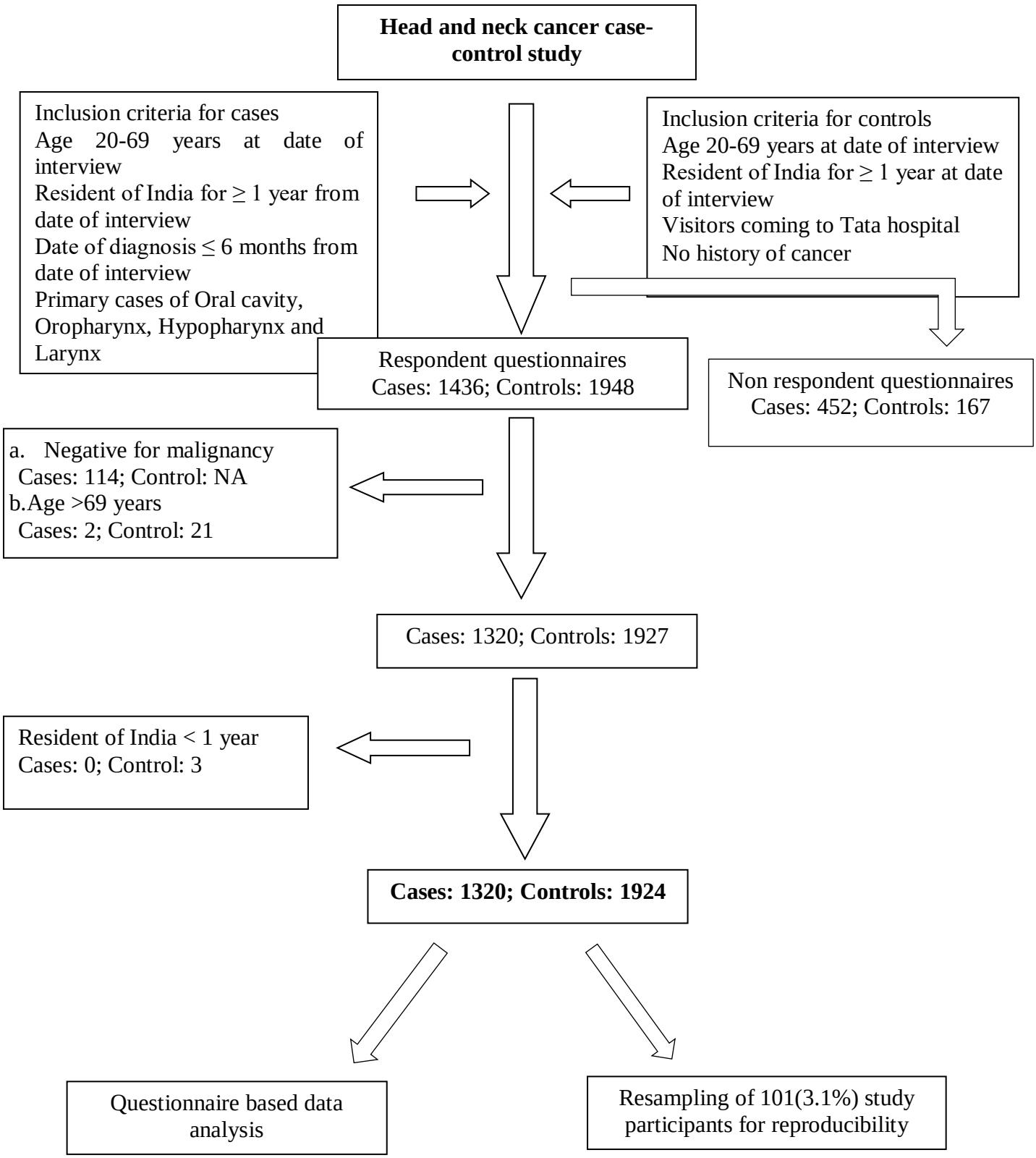

Figure 2. 1: Flowchart representing enrolment of study participants in head and neck case control study

2.4.1 Training of social investigators

For homogeneity in data collection social investigators were trained for questionnaire filling according to the instruction manual prepared for earlier case-control studies. The instruction manual contains detailed guidelines and figures wherever required for better understanding of questions by the social investigator as well as the respondent [36]. Instruction manual for data entry with predefined logical checks was used for training of data entry operators [37].

2.4.2 Monitoring of daily work

The forms were checked at three levels for completeness of information, first by the social investigator, immediately after taking the interview, second by the data manager, on following day of the interview and finally by the data entry operators, before entering the data. The questionnaire was checked daily for completeness of information.

2.4.3 Quality checks on data entry

Logical checks were prepared to identify errors in data entry.

2.4.4 Reproducibility questionnaire

Reproducibility questionnaire was designed containing constant variables (non changing with time) such as height, age, tobacco use, alcohol use, no. of pregnancies. The reproducibility questionnaire was completed for 101 study participants (approximately 3.1% of total study participants). The interval between main questionnaire and reproducibility questionnaire was minimum of 7 days. Details of the exposures measured are given in the table.

Table 2. 1: Reproducibility of measured exposure

Variable	Study mean N=101 (Reproducibility mean)	Coefficient of correlation (%)
Age	49.48 (49.47)	99.84
Tobacco Smoking	NA	97.47
Tobacco chewing	NA	100
Alcohol drinking	NA	92.83
Education	NA	70.09

2.4.5 Refresher training of social investigators

In order to keep optimum quality of the data refresher training of the social investigators was conducted biannually. The social investigators were trained according to instruction manual of questionnaire filling. Mock interviews of social investigators were also performed to assess correctness of the interview and questionnaire filling. In addition, weekly and monthly meetings were conducted to address the issues of data collection.

2.5 Exposure assessment

2.5.1 Tobacco smoking

All study participants were asked about tobacco smoking history (past and current) as a part of the questionnaire. A study participant was defined as a tobacco smoker if he has smoked atleast one cigarette/bidi per week or ≥ 50 cigarettes/bidi over a period of six months, whichever was earlier. When the study participant was identified as tobacco smoker according to study definition, detailed information was asked about tobacco smoking consumption pattern. Data

was collected on the type of tobacco smoked cigarette, bidi or others (chillum, chutta, hookah, etc), the age at initiation and age of quitting, number of cigarette/bidi smoked in a day. Information was collected accordingly if the study participant smoked tobacco at different time points in lifetime. A packet of cigarettes was defined as 10 cigarettes and a packet of bidi was defined as 25 bidis respectively.

Tobacco smoking association was evaluated with HNC, OC, OPX, HPX and LX by using different categories of tobacco smoking use such as “never vs. ever”, “manufactured cigarette use”, “bidi use” and “other type of smoking use”. “Any type of smoking” included different forms of smoking such as cigarette, bidi (thin cigarette filled with tobacco flake and wrapped in a tendu leaf tied with a string at one end), cheroot or stogie (cylindrical cigar with both ends clipped during manufacturing), cigar (tightly-rolled bundle of dried and fermented tobacco), water pipes/hookah (single or multi-stemmed, often glass-based instrument for smoking in which the smoke is cooled by water), roll your own (cigarette made from loose tobacco and rolling papers), chuttas (coarsely prepared cheroots), dhumti (conical cigar made by rolling tobacco leaf in the leaf of another plant), hooklis (clay pipe),chillum (straight conical pipe with end-to-end channel, traditionally made of clay).

The assessment of smoking habits was performed by methods followed and endorsed by IARC, validated, standardized and extensively used in broad epidemiologic studies within the International Head and Neck Cancer Epidemiology Consortium (INHANCE)[119],[21]. Association of tobacco smoking with HNC, OC, HPX and LX was evaluated by calculating pack years. For the calculation of pack-years, the amount of tobacco in grams was estimated as 1 per cigarette, 0.5 per bidi and 2 per cigar, cheroot and chutta. Cumulative pack years of a particular subject were calculated by addition of cigarette pack years, bidi pack years and other pack years

(if any) and multiplying them by duration of smoking. The pack years were then categorized in to quartiles according to consumption of controls and association was evaluated by assuming non-smokers as reference. Accordingly, tobacco smoking risk was evaluated for HNC, OC, OPX, HPX and LX. The risk was adjusted for confounding variables age (continuous variable), gender, region of residence at enrolment, socio-economic status, tobacco chewing and alcohol drinking.

2.5.2 Tobacco chewing

All study participants were asked about tobacco chewing history (past and current) as a part of the questionnaire. A study participant was defined as a tobacco chewer if he/she has chewed at least once a week for six months or more. When the study participant was identified as tobacco chewer according to study definition, detailed information was asked about tobacco chewing consumption pattern. Data was collected on chewed tobacco preparation and its constituents such as lime, areca nut, betel leaf and catechu. Information was collected on consumption of commercial preparations of tobacco such as *Gutka* (dry mixture of crushed areca nut, tobacco, catechu, lime, aroma, flavoring and other additives), *Khaini* (tobacco and slaked lime), *Mawa* (mixture of shaving of areca nut, scented tobacco, lime) and *masheri* (roasted, powdered preparation made by baking tobacco on a hot metal plate until it is uniformly black), *Lal dant manjan* (a red-coloured tooth powder. Traditionally, which contained tobacco but after the passage of a law in date banning the use of tobacco in dental care products, the listing of tobacco as an ingredient was stopped), *gul* (pyrolysed tobacco product), *gudhaku* (paste made of tobacco and molasses) ,creamy snuff (commercial preparations of tobacco paste marketed in toothpaste-like tubes which are advertised as possessing anti-bacterial activity and being good for the gums

and teeth) and products without tobacco such as only areca nut chewing and *Paan masala* (commercial preparation containing areca nut, slaked lime, catechu and condiments).

Association of different types of tobacco chewed with HNC, OC, OPX, HPX and LX was evaluated by "never vs. ever" tobacco chewer, *Betel quid* with tobacco (combination of betel leaf, areca nut, lime, catechu and tobacco), types of commercial tobacco use (*Gutka,Khaini,Mawa* and *Mishri)*, other tobacco products like *gul, gudhakhu*, *nash* and zarda, areca nut and *Betel quid* without tobacco use. The dose response relationship was assessed by calculating duration of chewing in years by taking non-chewers as reference and categorized into >0, >10 and >20 years. The risk was adjusted for confounding variables age (continuous variable), gender, region of residence at enrolment, socio-economic status, tobacco smoking and alcohol drinking.

We also performed analysis by categorizing the tobacco products into 'tobacco products with areca nut' and 'tobacco products without areca nut' according to WHO manual [120]. We also categorized tobacco products according to their method of preparation into commercially and manually adapting from reference [69],[65]

2.5.3 Alcohol drinking

All study participants were asked about alcohol history (past and current) as a part of the questionnaire. A study participant was defined as an alcohol drinker if he/she has consumed any type of alcoholic beverage at least once a week for six months or more. When the study participant was identified as alcohol drinker according to study definition, detailed information was asked about alcohol consumption pattern. The structured questionnaire included questions on type and frequency of alcohol consumption. Data was collected on beer (brewed by

fermenting malted barley), whisky (beverage made from fermentation of malted grains such wheat, maize, rye and oats), wine (beverage prepared by fermenting grape juice [white wine] or crushed grapes [red wine]), toddy (alcoholic beverage prepared from sap of various species of palm tree), country liquor (alcoholic beverage prepared by fermenting molasses of sugarcane) and other alcoholic drinks such as rum, brandy and vodka.

The frequency of alcoholic consumption was collected in units/day where in each bottle unit of alcoholic beverage equals to 750ml and where 4 glasses=1 bottle, 2 cans=1 bottle, 15 shots=1 bottle (1 shot=50ml).

Similar to tobacco smoking, assessment of drinking habits was performed by methods followed by IARC, validated, standardized and extensively used in broad epidemiologic studies such as International Head and Neck Cancer Epidemiology Consortium (INHANCE) [119],[21].

Alcohol drinking was converted into grams of ethanol, considering that one litre of ethanol weighs 798 g and that beer contains 5% ethanol in volume; wine 12%; liqueurs 30% and distilled spirits 41% (Table 2.2). Cumulative exposure to alcohol was expressed in gram-years (grams of ethanol consumed daily multiplied by the number of drinking years). Gram years of ethanol were then categorized into quartiles to assess the dose response relationship between alcohol and HNC and primary sub-sites OC, OPX, HPX and LX. For statistical analysis to measure risk of alcohol consumption never alcohol drinkers were taken as reference.

Table 2. 2: Ethanol content in various types of alcoholic beverage calculated from reference [108]

Type of alcoholic beverage	Ethanol content in % v/v	Ethanol content in gms/ml
Beer	5	0.039
Whisky	41	0.327
Country liquor	41	0.327
Toddy	5	0.039
Wine	12	0.095
Vodka, gin, brandy and rum	41	0.327

2.5.4 Socio-economic status

Completed highest level of education was used as proxy variable indicator of socio-economic status. No education or illiterate was used as reference. Education was categorized into literate, < 5 years of schooling, 5-8 years of schooling, High school, college graduation and above. Association of socio-economic status with HNC, OC, OPX, HPX, and LX was analyzed. The statistical association was adjusted for confounding variables age (continuous), gender, region of residence (North, East, West, Central, and South), tobacco smoking, tobacco chewing and alcohol drinking.

2.6 Statistical analysis

Crude and adjusted Odds ratio and their 95% CI for developing HNC, OC, OPX, HPX and LX were calculated for tobacco smoking, tobacco chewing, alcohol drinking and socio-economic status. Unconditional logistic regression models were adjusted for potential confounders such as age (continuous variable), region of residence (North, South, East, West and Central India),

gender, socio-economic status (literate, < 5 years of schooling, 5-8 years of schooling, High school, college graduation and above). Models estimating risk of tobacco chewing were additionally adjusted for tobacco smoking (never/ever) and alcohol drinking (never/ever), while models assessing tobacco smoking were adjusted for tobacco chewing (never/ever) alcohol drinking (never/ever). Similarly, models estimating risk of alcohol drinking were additionally adjusted for tobacco chewing (never/ever) and tobacco smoking (never/ever). Models estimating risk of socio-economic status were additionally adjusted for tobacco chewing (never/ever), tobacco smoking (never/ever) and alcohol drinking (never/ever). Study participants for whom values for one or more of the variables in the models were missing were eliminated from the analyses. Test for linear trend for ordered variables were performed by assigning the score j to the j^{th} exposure level of a categorical variable (where j = 1, 2 . . .) and treating it as a continuous predictor in unconditional logistic regression. Test for heterogeneity to estimate differences in stratum specific odds ratio for HNC subsites (OC, OPX,HPX and LX) and types/products of tobacco smoking, tobacco chewing and alcohol drinking was performed by using multinomial regression and Wald test testing the null hypothesis that the risk associated with the exposure was same across all sub-types.

2.6.1 Effect modification

Joint associations of tobacco and alcohol were studied in relation to HNC, OC, OPX, HPX and LX. Four categories of smoking pack years were used (≤24, 25-66, 67-216, ≥216) according to consumption reported by study controls. Three categories of cumulative chew years were used (≤100, 100-200, ≥ 300 chew years). Gram years of alcohol were divided into 4 categories (≤548.1, 548.1-3380.31, 3380.81-9871.31, ≥9871.313). To analyze the interaction effect, interaction term approach was used which is widely accepted. The terms generated to study

smoking and drinking interaction were named as “level 1 drinker, level 1 smoker, level 1 drinker level 2 smoker, level 2 drinker level 1 smoker, level 2 drinker level 2 smoker, level 3 drinker level 1 smoker, level 3 drinker level 2 smoker, level 4 drinker level 1 smoker, level 4 drinker level 2 smoker” according to the categories of pack years and gram years of controls exposure. The terms generated to study chewing and drinking interaction were named as “level 1 chewer, level 2 chewer, level 3 chewer, level 4 chewer, level 1 drinker level 1 chewer, level 1 drinker level 2 chewer, level 1 drinker level 3 chewer, level 2 drinker level 1 chewer, level 2 drinker level 2 chewer, level 2 drinker level 3 chewer, level 3 drinker level 1 chewer, level 3 drinker level 2 chewer, level 3 drinker level 3 chewer, level 4 drinker level 1 chewer, level 4 drinker level 2 chewer, level 4 drinker level 3 chewer” Never smoker-never drinker and never chewer-never drinker were used as a reference category to assess the joint association.

2.7 Results

A total of 1320 cases and 1924 controls were enrolled in the study. Out of 1320 cases 950 (71.96%) were oral cavity, 166 (12.57%) were Oropharynx, 117 (8.86%) were hypopharynx and 86 (6.51%) were larynx cases. Distribution of cases and controls with respect to age and region of residence at enrolment, gender, tobacco use, alcohol use and education are given in Table 2.3. The mean age at enrolment of cases and controls was 48.43 and 46.23 respectively. Ever tobacco chewers were higher in cases than controls, the highest proportion being in oral cavity cases (80.95%). Ever tobacco smokers were highest in Oropharynx cases (69.28%) compared to controls (18.56%). Ever alcohol drinkers were highest in Oropharynx cases (32.53%) compared to controls (10.55%). Controls had higher proportion of college graduates (35.03%) as compared to cases- oral cavity (21.79%), Oropharynx (9.64%), hypopharynx (16.24%) and larynx (12.79%). Maximum oral cavity cases were from west region (38.21%), Oropharynx cases were

from north region (34.94%), hypopharynx cases were from west region (40.17) and larynx cases were from west region (37.21%). Maximum controls were from east region (31.44%).

2.7.1 Tobacco smoking

A statistically significant risk was observed in HNC cases for 'ever' smokers. Highest risk was observed for Oropharynx cancer (OR=6.0; 95% CI: 4.0-9.0) after adjusting for potential confounders like age (continuous), gender, region of residence, gender, socio-economic status, tobacco chewing and alcohol drinking. The risk of ever smoking for overall HNC was 2.3 (95% CI: 1.9-2.8). Risk for bidi smoking was observed to be higher for all HNC sub-sites than manufactured cigarette smoking. Bidi smoking risk was highest for OPX cancer (OR=11.6; 95% CI= 7.4-18.2; p value for heterogeneity <0.001). Manufactured cigarette smoking risk was highest for HPX (OR=3.9; 95% CI: 2.3-6.5; p value for heterogeneity <0.001). Cumulative exposure for tobacco smoking was measured in smoking pack years. The pack years were categorized into quartiles according to study controls consumption. Risk for OC, OPX, HPX and LX cancer increased with increase in pack years consumption, the risk being highest for last quartile. A dose response relationship was observed between pack years consumption and risk of HNC sub-sites (Table 2.4.2). In analysis of joint association of cigarette and bidi smoking risk was observed for all HNC sub-sites. The joint association risk was highest for OPX (OR=7.2; 95% CI: 3.7-13.8; p for interaction: <0.001) (Table 2.5.2).

2.7.2 Tobacco chewing

A statistically significant risk was observed for 'ever' tobacco chewers. Highest risk was observed for OC cancer (OR=8.7; 95% CI: 7.1-10.7) after adjusting for potential confounders like age (continuous), gender, region of residence, gender, socio-economic status, tobacco

smoking and alcohol drinking. Risk for types of tobacco products with HNC sub-sites was also analyzed. Chewing *Betel quid* with tobacco, tobacco quid, *Gutka, Khaini, Mawa, Mishri* had highest risk for OC cancer after adjusting for potential confounders (p value for heterogeneity <0.05) (Table 2.6.2). Amongst all types of tobacco products chewed, *Gutka* chewing had highest risk for OC cancer (OR=28.09; 95% CI: 20.2-39.0; p value for heterogeneity <0.05). Chewing areca nut, betel leaf or *Paan masala* without tobacco also had highest risk for OC cancer (OR=6.3; 95% CI: 3.2-12.2; p value for heterogeneity >0.05) (Table 2.6.2). In analysis to tobacco chewing duration and its risk for HNC, highest risk was observed for 11-20 years of chewing for overall HNC (Table 2.7.2). In analysis of tobacco chewing frequency per day, highest risk was observed for chewing 11-20 times/day for HNC. Analysis was performed to assess risk of cumulative exposure of chewing (number of times chewed per day X duration of chewing in a lifetime). Risk for HNC, OC, OPX, HPX and LX increased with increase in cumulative years of chewing. Analysis of risk of chewing duration and frequency on OC cancer was stratified by types of tobacco product (Table 2.8.2 and 2.9.2). *Gutka* chewing had highest risk for increase in chewing duration and frequency for OC cancer (Table 2.8.2) after adjusting for potential confounders (p value for heterogeneity <0.05).

We observed higher risk for tobacco products containing areca nut for OC, LX and HNC (p value for heterogeneity >0.05) (Table 2.10.2). We also observed higher risk for commercially prepared tobacco products (p value for heterogeneity <0.05) (Table 2.11.2)

2.7.3 Alcohol drinking

A statistically significant risk was observed for 'ever' alcohol drinkers for HNC sub-sites. Highest risk of ever alcohol drinking was observed for OPX cancer (OR=1.8; 95% CI: 1.2-2.8;

p value for heterogeneity >0.05) after adjusting for potential confounders like age (continuous), gender, region of residence, gender, socio-economic status, tobacco smoking and tobacco chewing. In analysis of risk for types of alcohol drinks, drinking country spirit had highest risk for OPX cancer (OR=2.5; 95% CI: 1.4-4.4; p value for heterogeneity >0.05). Statistically significant risk was observed for drinking beer, whisky, country spirit and toddy (p value for heterogeneity >0.05) (Table 2.12.2). Analysis of alcohol drinking duration also showed risk for HNC sub-sites especially for OPX (p value for heterogeneity >0.05) (Table 2.13.2). Cumulative exposure of alcohol drinking was measured by ethanol gram years. Increase in risk of OC, OPX, HPX and overall HNC cancer was observed for increase in ethanol gram years (Table 2.13.2). Ethanol gram years analysis stratified by alcohol types was also performed, which showed increase in risk for increase in gram years. For interpretation purposes the gram years categorized were equivalent to consuming 120ml/day for year alcohol drink respectively (Table 2.14.2).

2.7.4 Joint association between tobacco and alcohol drinking

A statistically significant interaction was obtained between highest quartiles of smoking pack years and ethanol gram years for OC and HNC (Table 2.15. 1). For OC we got joint risk of 8.2 (95% CI: 1.9-35.0) and for HNC we got 18.1 (95% CI: 5.2-62.8). We observed more than multiplicative interaction between smoking and alcohol as observed in previous studies. We could not asses interaction for OPX, HPX and LX due to limited sample size in the strata.

A statistically significant interaction was obtained between highest levels of chew years and highest quartiles of ethanol gram years for OC and HNC (Table 2.15. 4). For OC we observed joint risk of 14.6 (95% CI: 6.1-35.0) and for HNC we observed risk of 10.5 (95% CI: 4.7-23.5).

We observed more than multiplicative interaction between chewing and alcohol. We could not asses interaction for OPX, HPX and LX due to limited sample size in the strata.

2.7.5 Socio-economic status

Completed education of the subject was used as proxy variable for socio-economic status. A statistically significant protective association was observed between literacy and above education for OC and overall HNC after adjusting for potential confounders like age (continuous), gender, region of residence, tobacco smoking pack years, tobacco chewing (ever/never) and alcohol gram years (Table 2.16. 2). For OPX, HPX and LX a statistically significant protective association was obtained after high school and above (p value for heterogeneity <0.05).

Table 2. 3: Characteristics of study participants in head and neck case control study.

Parameters	Categories	Cases (N=1320)								Controls (N=1924)		p value
		Oral cavity (N=950)		Oropharynx (N=166)		Hypopharynx (N=117)		Larynx (N=86)				
		Number	%	Number	%	Number	%	Number	%	Number	%	
Age at Enrolment	18-29	43	4.53	2	1.2	2	1.71	1	1.16	125	6.5	0.55
	30-39	235	24.74	9	5.42	6	5.13	6	6.98	397	20.6	
	40-49	320	33.68	37	22.29	23	19.66	9	10.47	598	31.08	
	50-59	221	23.26	62	37.35	36	30.77	34	39.53	506	26.3	
	60-69	131	13.79	56	33.73	50	42.74	36	41.86	298	15.49	
	Mean(±SD)	48.43(±10.7)								46.23(±11.06)		
Region of residence at enrolment	North	338	35.58	58	34.94	23	19.66	21	24.42	573	29.78	0.12
	West	363	38.21	42	25.3	47	40.17	32	37.21	582	30.25	
	South	8	0.84	1	0.6	2	1.71	0	0	17	0.88	
	East	166	17.47	35	21.08	40	34.19	25	29.07	605	31.44	
	Central	73	7.68	30	18.07	5	4.27	8	9.3	143	7.43	
	Missing	2	0.21	0	0	0	0	0	0	4	0.21	
Gender	Males	827	87.05	150	90.36	104	88.89	81	94.19	1207	62.73	<0.001
	Females	123	12.95	16	9.64	13	11.11	5	5.81	717	37.27	
Any tobacco use	Never	113	11.89	13	7.83	17	14.53	13	15.12	1187	61.69	<0.001
	Ever	837	88.11	153	92.17	100	85.47	73	84.88	737	38.31	
Tobacco chewing	Never	181	19.05	75	45.18	48	41.03	42	48.84	1399	72.71	<0.001
	Ever	769	80.95	91	54.82	69	58.97	44	51.16	525	27.29	
Tobacco smoking	Never	648	68.21	51	30.72	42	35.9	31	36.05	1567	81.44	<0.001
	Ever	302	31.79	115	69.28	75	64.1	55	63.95	357	18.56	
Alcohol drinking	Never	695	73.16	112	67.47	87	74.36	70	81.4	1721	89.45	<0.001
	Ever	255	26.84	54	32.53	30	25.64	16	18.6	203	10.55	
Education	Nil, Illiterate	98	10.32	21	12.65	12	10.26	9	10.47	124	6.44	0.01
	Literate	25	2.63	10	6.02	5	4.27	4	4.65	58	3.01	
	< 5 years of schooling	73	7.68	16	9.64	15	12.82	10	11.63	93	4.83	
	5-8 years of schooling	248	26.11	60	36.14	34	29.06	28	32.56	369	19.18	
	High School	298	31.37	43	25.9	32	27.35	24	27.91	606	31.5	
	College graduation or more	207	21.79	16	9.64	19	16.24	11	12.79	674	35.03	
	Missing	1	0.11	0	0	0	0	0	0	0	0	

Table 2.4. 1: Association of tobacco smoking with different head and neck cancer sub-sites adjusted for age, gender, region and socio-economic status.

Parameter	HNC Primary Sub-site												All HNC cases(N=1320)		
	Oral cavity (N=950)			Oropharynx(N=166)			Hypopharynx(N=177)			Larynx(N=86)					
	Ca/Co	OR (95%CI)	p value	Ca/Co	OR (95%CI)	p value	Ca/Co	OR (95%CI)	p value	Ca/Co	OR (95%CI)	p value	Ca/Co	OR (95%CI)	p value
Never smoker	648/1567	Reference		51/1567	Reference		42/1567	Reference		31/1567	Reference		773/1567	Reference	
Ever smoker	302/357	1.4(1.1-1.7)	<0.001	115/357	5.68(3.8-8.4)	<0.001	75/357	4.55(2.9-7.1)	<0.001	55/357	3.55(2.1-5.8)	<0.001	547/357	2.0(1.6-2.3)	<0.001
Type of tobacco smoked															
Manufactured cigarettes	196/1567	1.3(1.07-1.6)	0.009	42/1567	3.2(1.9-5.0)	<0.001	42/1567	3.9(2.4-6.6)	<0.001	19/1567	1.8(1.0-3.4)	0.05	299/1567	1.6(1.3-2.0)	<0.001
Bidi	157/1567	1.6(1.2-2.1)	<0.001	91/1567	10.2(6.6-15.8)	<0.001	42/1567	5.3(3.1-9.0)	<0.001	42/1567	6.4(3.6-11.1)	<0.001	326/1567	2.7(2.1-3.4)	<0.001
Number of pack years															
0 (Reference)	648/1567	Reference		51/1567	Reference		42/1567	Reference		31/1567	Reference		773/1567	Reference	
Q1(12,12,13)[a]	72/90	1.3(0.9-1.8)	0.12	4/90	0.9(0.33-2.8)	0.98	5/90	1.6(0.6-4.4)	0.33	3/90	1.1(0.3-3.8)	0.86	84/90	1.3(0.9-1.8)	0.09
Q2(44.2,42,20)	74/88	1.4(1.0-1.9)	0.04	13/88	2.7(1.3-5.3)	0.004	4/88	0.9(0.3-2.7)	0.88	7/88	1.7(0.7-4.2)	0.23	98/88	1.5(1.1-2.1)	0.006
Q3(129.3,120,75)	90/88	1.7(1.2-2.3)	0.001	42/88	8.8(5.3-14.6)	<0.001	31/88	7.4(4.2-13.0)	<0.001	19/88	4.7(2.4-9.0)	<0.001	182/88	2.7(2.0-3.6)	<0.001
Q4(605,438.7,386.2)	61/88	1.1(0.8-1.7)	0.36	53/88	9.2(5.6-15.3)	<0.001	34/88	7.3(4.2-12.9)	<0.001	24/88	5.5(2.9-10.2)	<0.001	172/88	2.4(1.8-3.2)	<0.001
P_{trend}	0.002			<0.001			<0.001			<0.001			<0.001		
Risk for every 500 pack years increase	1.05(0.83-1.3)		0.65	2.3(1.7-3.1)		<0.001	2.3(1.6-3.1)		<0.001	1.6(1.1-2.2)		0.006	1.6(1.2-2.0)		<0.001

Abbreviations: CI: Confidence Interval; OR: Odds Ratio

Ca/Co: Case/Control; All OR's are adjusted for age (continuous) , gender, region and socio-economic status

[a]Quartiles of tobacco smoking pack years based on study controls consumption. The values in the parenthesis are mean, median and inter-quartile range respectively.

Missing values are excluded from the analysis

Table 2.4. 2: Association of tobacco smoking with different head and neck cancer sub-sites adjusted for age, gender, region, socio-economic status, tobacco chewing and alcohol gram years.

Parameter	HNC Primary Sub-site												All HNC cases(N=1320)		
	Oral cavity (N=950)			Oropharynx(N=166)			Hypopharynx(N=177)			Larynx(N=86)					
	Ca/Co	OR (95%CI)	p value	Ca/Co	OR (95%CI)	p value	Ca/Co	OR (95%CI)	p value	Ca/Co	OR (95%CI)	p value	Ca/Co	OR (95%CI)	p value
Never smoker	648/1567	Reference		51/1567	Reference		42/1567	Reference		31/1567	Reference		773/1567	Reference	
Ever smoker	302/357	1.44(1.1-1.8)	<0.001	115/357	6.0(4.0-9.0)	<0.001	75/357	4.6(2.9-7.2)	<0.001	55/357	3.6(2.2-5.9)	<0.001	547/357	2.3(1.9-2.8)	<0.001
Type of tobacco smoked															
Manufactured cigarettes	196/1567	1.28(1-1.6)	0.04	42/1567	3.1(1.9-5.1)	<0.001	42/1567	3.9(2.3-6.5)	<0.001	19/1567	1.7(0.9-3.3)	0.06	299/1567	1.7(1.3-2.1)	<0.001
Bidi	157/1567	2.0(1.4-2.7)	<0.001	91/1567	11.6(7.4-18.2)	<0.001	42/1567	5.5(3.2-9.4)	<0.001	42/1567	6.6(3.8-11.5)	<0.001	326/1567	4.0(3.1-5.3)	<0.001
Number of pack years															
0 (Reference)	648/1567	Reference		51/1567	Reference		42/1567	Reference		31/1567	Reference		773/1567	Reference	
Q1(12,12,13)[a]	72/90	1.08(0.7-1.5)	0.65	4/90	0.9(0.3-2.8)	0.8	5/90	1.5(0.5-4.3)	0.38	3/90	1.1(0.3-3.8)	0.86	84/90	1.1(0.8-1.6)	0.35
Q2(44.2,42,20)	74/88	1.2(0.8-1.8)	0.21	13/88	2.8(1.4-5.7)	0.003	4/88	0.9(0.3-2.7)	0.88	7/88	1.7(0.6-4.1)	0.24	98/88	1.5(1.0-2.1)	0.01
Q3(129.3,120,75)	90/88	2.2(1.5-3.2)	<0.001	42/88	9.4(5.6-15.8)	<0.001	31/88	7.1(4-12.7)	<0.001	19/88	4.8(2.5-9.3)	<0.001	182/88	3.8(2.7-5.2)	<0.001
Q4(605,438.7,386.2)	61/88	1.6(1-2.4)	0.02	53/88	10.4(6.2-17.5)	<0.001	34/88	8.2(4.6-14.6)	<0.001	24/88	5.7(3-10.6)	<0.001	172/88	3.9(2.8-5.4)	<0.001
P_{trend}	<0.001			<0.001			<0.001			<0.001			<0.001		
Risk for every 500 pack years increase	1.2(0.9-1.6)		0.15	2.4(1.8-3.3)		<0.001	2.5(1.7-3.5)		<0.001	1.6(1.1-2.3)		0.005	2.1(1.6-2.7)		<0.001

Abbreviations: CI: Confidence Interval; OR: Odds Ratio

Ca/Co: Case/Control; All OR's are adjusted for age (continuous), gender, region, socio-economic status, tobacco chewing and alcohol gram years.

[a]Quartiles of tobacco smoking pack years based on study controls consumption. The values in the parenthesis are mean, median and inter-quartile range respectively.

Missing values are excluded from the analysis

Table 2.5. 1: Estimates of odds ratio of joint association of cigarette and bidi on Oral cavity cancer.

HNC Primary sub-site	Bidi Smoking	Cigarette smoking				
		No		Yes		p for interaction
		Ca/Co	OR (95%CI)	Ca/Co	OR (95%CI)	
Oral cavity (N=950)	No	650/1568	Reference	149/223	1.2(0.9-1.6)	0.22
	Yes	104/87	2.2(1.5-3.2)	47/46	1.7(1.1-2.9)	

Abbreviations: CI: Confidence Interval; OR: Odds Ratio

Ca/Co: Case/Control; All OR's are adjusted for age (continuous), gender, region, socio-economic status, tobacco chewing and alcohol gram years.

Missing values are excluded from the analysis

Table 2.5. 2: Estimates of odds ratio of joint association of cigarette and bidi smoking on Oropharynx cancer.

HNC Primary sub-site	Bidi Smoking	Cigarette smoking				
		No		Yes		p for interaction
		Ca/Co	OR (95%CI)	Ca/Co	OR (95%CI)	
Oropharynx (N=166)	No	53/1658	Reference	22/223	2.1(1.1-3.7)	0.005
	Yes	71/87	13.7(8.4-22.5)	20/46	7.1(3.7-13.8)	

Abbreviations: CI: Confidence Interval; OR: Odds Ratio

Ca/Co: Case/Control; All OR's are adjusted for age (continuous), gender, region, socio-economic status, tobacco chewing and alcohol gram years.

Missing values are excluded from the analysis.

Table 2.5. 3: Estimates of odds ratio of joint association of cigarette and bidi smoking on Hypopharynx cancer.

HNC Primary sub-site	Bidi Smoking	Cigarette smoking				
		No		Yes		p for interaction
		Ca/Co	OR (95%CI)	Ca/Co	OR (95%CI)	
Hypopharynx (N=177)	No	43/1568	Reference	32/223	3.8(2.2-6.5)	0.001
	Yes	32/87	6.3(3.5-11.2)	10/46	4.2(1.9-9.5)	

Abbreviations: CI: Confidence Interval; OR: Odds Ratio

Ca/Co: Case/Control; All OR's are adjusted for age (continuous), gender, region, socio-economic status, tobacco chewing and alcohol gram years.

Missing values are excluded from the analysis.

Table 2.5. 4: Estimates of odds ratio of joint association of cigarette and bidi smoking on Larynx cancer.

HNC Primary sub-site	Bidi Smoking	Cigarette smoking				p for interaction
		No		Yes		
		Ca/Co	OR (95%CI)	Ca/Co	OR (95%CI)	
Larynx (N=86)	No	31/1568	Reference	13/223	1.5(0.7-3.0)	0.01
	Yes	36/87	8,5(4.6-15.5)	6/46	2.7(1.0-7.4)	

Abbreviations: CI: Confidence Interval; OR: Odds Ratio

Ca/Co: Case/Control; All OR's are adjusted for age (continuous), gender, region, socio-economic status, tobacco chewing and alcohol gram years.

Missing values are excluded from the analysis.

Table 2.5. 5: Estimates of odds ratio of joint association of cigarette and bidi smoking on overall head and neck cancer.

HNC Primary sub-site	Bidi Smoking	Cigarette smoking				p for interaction
		No		Yes		
		Ca/Co	OR (95%CI)	Ca/Co	OR (95%CI)	
All HNC cases (N=1320)	No	778/1568	Reference	216/223	1.5(1.2-2.0)	<0.001
	Yes	243/87	5.1(3.7-7.0)	83/46	2.6(1.7-3.9)	

Abbreviations: CI: Confidence Interval; OR: Odds Ratio

Ca/Co: Case/Control; All OR's are adjusted for age (continuous), gender, region, socio-economic status, tobacco chewing and alcohol gram years.

Missing values are excluded from the analysis.

Table 2.6. 1: Association of tobacco chewing with head and neck cancer sub-sites adjusted for age, gender, region and SES.

Parameter	Categories	HNC Primary sub-site												All HNC cases(N=1320)		
		Oral cavity (N=950)			Oropharynx(N=166)			Hypopharynx(N=177)			Larynx(N=86)					
		Ca/Co	OR (95%CI)	p value	Ca/Co	OR (95%CI)	p value	Ca/Co	OR (95%CI)	p value	Ca/Co	OR (95%CI)	p value	Ca/Co	OR (95%CI)	p value
Tobacco chewing	Never	181/1399	Reference		75/1399	Reference		48/1399	Reference		42/1399	Reference		346/1399	Reference	
	Ever	779/525	8.7 (7.1-10.7)	<0.001	91/525	1.9 (1.3-2.6)	<0.001	69/525	2.3 (1.5-3.5)	<0.001	44/525	1.4 (0.91-2.3)	0.11	974/525	5.5 (4.6-6.4)	<0.001
Type of tobacco chewing	BQ+T[a]	189/1399	12.1 (8.8-16.5)	<0.001	27/1399	2.6 (1.5-4.5)	<0.001	22/1399	3.5 (1.9-6.4)	<0.001	12/1399	2.0 (0.99-4.1)	0.05	242/1399	7.6 (5.7-10.1)	<0.001
	Tobacco quid[b]	368/1399	16.7 (12.8-21.9)	<0.001	40/1399	3.2 (2.0-5.1)	<0.001	33/1399	3.9 (2.3-6.6)	<0.001	23/1399	3.1 (1.7-5.5)	<0.001	464/1399	10.5 (8.2-13.3)	<0.001
	Gutka	306/1399	28.3 (20.3-39.2)	<0.001	17/1399	4.6 (2.3-9.0)	<0.001	8/1399	3.1 (1.3-7.1)	<0.001	4/1399	1.8 (0.5-5.5)	0.3	336/1399	18.5 (13.6-25.3)	<0.001
	Khaini	94/1399	11.0 (7.4-16.3)	<0.001	12/1399	2.0 (0.99-4.3)	0.05	11/1399	3.1 (1.4-6.7)	0.004	3/1399	0.92 (0.26-3.2)	0.91	120/1399	7.0 (4.8-10.2)	<0.001
	Mawa	49/1399	7.3 (4.5-11.6)	<0.001	9/1399	2.1 (0.94-4.8)	0.07	3/1399	0.99 (0.28-3.4)	0.99	2/1399	0.78(0.17-3.4)	0.74	63/1399	4.5 (2.9-7.0)	<0.001
	Mishri	35/1399	5.8 (3.5-9.7)	<0.001	3/1399	1.3 (0.35-4.6)	0.7	9/1399	5.8 (2.4-13.6)	<0.001	2/1399	1.4 (0.29-6.8)	0.66	49/1399	4.5 (2.7-7.2)	<0.001
Others with tobacco[c]		39/1399	22.7 (11.5-44.9)	<0.001	5/1399	9.1 (2.8-29.5)	<0.001	No observations			4/1399	10.0 (2.6-39.1)	<0.001	49/1399	16.4 (8.4-31.9)	<0.001
Other without tobacco[d]		21/1399	7.0(3.7-13.4)	<0.001	7/1399	5.8 (2.2-15.6)	<0.001	4/1399	4.7 (1.4-15.6)	0.1	2/1399	1.93 (0.39-9.5)	0.42	34/1399	5.7 (3.2-10.3)	<0.001

Abbreviations: CI: Confidence Interval; OR: Odds Ratio

Ca/Co: Case/Control; All OR's are adjusted for age (continuous), gender, region and socio-economic status.

[a]Betel leaf with tobacco and/ or *areca nut and* or lime and/or *catachu.*

[b]Chewing tobacco with lime and/or *areca nut and* or *catachu* without betel leaf.

[c]Chewing or application of *gul and/or gudhakhu and/or naash* and/or *zarda*.

[d]Chewing of *areca nut* and/or betel leaf and/or *Paan masala* without tobacco.

Missing values are excluded from the analysis.

Table 2.6. 2: Association of tobacco chewing with head and neck cancer sub-sites adjusted for age, gender, region, SES, tobacco smoking pack years and alcohol gram years.

Parameter	Categories	HNC Primary sub-site												All HNC cases(N=1320)		
		Oral cavity (N=950)			Oropharynx(N=166)			Hypopharynx(N=177)			Larynx(N=86)					
		Ca/Co	OR (95%CI)	p value	Ca/Co	OR (95%CI)	p value	Ca/Co	OR (95%CI)	p value	Ca/Co	OR (95%CI)	p value	Ca/Co	OR (95%CI)	p value
Tobacco chewing	Never	181/1399	Reference		75/1399	Reference		48/1399	Reference		42/1399	Reference		346/1399	Reference	
	Ever	779/525	8.7 (7.1-10.7)	<0.001	91/525	2.0 (1.3-2.9)	<0.001	69/525	2.4 (1.6-3.8)	<0.001	44/525	1.5 (0.9-2.4)	0.09	974/525	5.8 (4.8-6.9)	<0.001
Type of tobacco chewing	BQ+T[a]	189/1399	12.2 (8.9-16.7)	<0.001	27/1399	3.0 (1.7-5.3)	<0.001	22/1399	3.9 (2.1-7.4)	<0.001	12/1399	2.1 (1.0-4.4)	0.03	242/1399	8.2 (6.1-10.9)	<0.001
	Tobacco quid[b]	368/1399	16.9 (12.9-22.1)	<0.001	40/1399	3.5 (2.1-5.7)	<0.001	33/1399	4.2 (2.4-7.3)	<0.001	23/1399	3.1 (1.7-5.7)	<0.001	464/1399	11.4 (8.9-14.6)	<0.001
	Gutka	306/1399	28.09 (20.2-39)	<0.001	17/1399	4.2 (2.0-8.5)	<0.001	8/1399	3.0 (1.2-7.4)	0.01	4/1399	1.5 (0.4-5.2)	0.47	336/1399	18.9 (13.8-25.9)	<0.001
	Khaini	94/1399	11.0 (7.4-16.5)	<0.001	12/1399	2.3 (1.1-5.1)	0.02	11/1399	4.0 (1.8-8.9)	0.001	3/1399	0.9 (0.2-3.4)	0.98	120/1399	7.6 (5.2-11.2)	<0.001
	Mawa	49/1399	7.3 (4.5-11.7)	<0.001	9/1399	2.2 (0.9-5.2)	0.06	3/1399	0.9 (0.2-3.8)	1.00	2/1399	0.8 (0.1-3.7)	0.81	63/1399	4.7 (3.0-7.3)	<0.001
	Mishri	35/1399	5.8 (3.5-9.7)	<0.001	3/1399	1.5 (0.4-5.6)	0.47	9/1399	5.8 (2.3-14.3)	<0.001	2/1399	1.5 (0.3-7.2)	0.61	49/1399	4.6 (2.8-7.5)	<0.001
Others with tobacco[c]		39/1399	22.1 (11.1-43.9)	<0.001	5/1399	8.5 (2.7-27.1)	<0.001	No observations			4/1399	10.0 (2.6-38.3)	0.001	49/1399	16.3 (8.3-31.8)	<0.001
Others without tobacco[d]		21/1399	6.3(3.2-12.2)	<0.001	7/1399	5.4 (1.9-15.2)	0.001	4/1399	5.4 (1.6-17.9)	0.006	2/1399	2.0 (0.4-9.9)	0.39	34/1399	5.3 (2.9-9.8)	<0.001

Abbreviations: CI: Confidence Interval; OR: Odds Ratio

Ca/Co: Case/Control; All OR's are adjusted for age (continuous), gender, region and socio-economic status, tobacco smoking pack years and alcohol gram years.

[a]Betel leaf with tobacco and/ or *areca nut* and/or lime and/or *catachu.*

[b]Chewing tobacco with lime and/or *areca nut* and/or *catachu* without betel leaf.

[c]Chewing or application of *gul and/or gudhakhu* and/or *naash* and/or *zarda.*

[d]Chewing of *areca nut* and/or betel leaf and/or *Paan masala* without tobacco.

Table 2.7. 1: Association of tobacco chewing by duration and frequency with sub-sites of head and neck cancer adjusted for age, gender, region and SES.

Parameter	Categories	HNC Primary sub-site												All HNC cases(N=1320)		
		Oral cavity (N=950)			Oropharynx(N=166)			Hypopharynx(N=177)			Larynx(N=86)					
		Ca/Co	OR (95%CI)	p value	Ca/Co	OR (95%CI)	p value	Ca/Co	OR (95%CI)	p value	Ca/Co	OR (95%CI)	p value	Ca/Co	OR (95%CI)	p value
Chewing duration (in years)	0	181/1399	Reference		75/1399	Reference		48/1399	Reference		42/1399	Reference		346/1399	Reference	
	1-5	100/99	6.2(4.4-8.5)	<0.001	12/99	1.8(0.9-3.5)	0.10	8/99	2.1(0.9-4.5)	0.08	4/99	1.1(0.4-3.3)	0.81	124/99	4.3(3.1-5.7)	<0.001
	6-10	152/87	10.2(7.4-13.9)	<0.001	8/87	1.3(0.9-2.8)	0.52	6/87	1.5(0.6-3.8)	0.35	7/87	1.7(0.7-4.0)	0.24	173/87	6.5(4.8-8.7)	<0.001
	>10	515/337	9.2(7.4-11.5)	<0.001	71/337	2.0(1.3-2.9)	<0.001	55/337	2.5(1.6-3.9)	<0.001	32/337	1.4(0.8-2.4)	0.16	674/337	5.5(4.5-6.6)	<0.001
P_{trend}			<0.001			<0.001			<0.001			0.13			<0.001	
Number of times chewed per day	0	181/1399	Reference		75/1399	Reference		48/1399	Reference		42/1399	Reference		346/1399	Reference	
	1-10	658/476	8.3(6.7-10.2)	<0.001	80/476	1.8(1.2-2.5)	0.001	61/476	2.2(1.4-3.4)	<0.001	42/476	1.5(0.95-2.4)	0.07	842/476	5.2(4.3-6.1)	<0.001
	11-20	96/38	14.8(9.7-22.5)	<0.001	9/38	2.9(1.2-6.6)	0.01	5/38	2.5(0.92-7.2)	0.07	0/38	NE	NE	110/38	8.5(5.7-12.7)	<0.001
	>20	10/7	8.1(1.9-22.3)	<0.001	2/7	3.5(0.65-18.9)	0.14	2/7	5.5(1.0-28.9)	0.04	1/7	3.3(0.38-28.9)	0.27	15/7	6.4(2.5-16.2)	<0.001
P_{trend}			<0.001			<0.001			<0.001			0.22			<0.001	
Risk for every 5 number of times chewed per day increase		1.3(1.1-1.4)		<0.001	1.2(1.0-1.4)		0.04	1.1(0.90-1.5)		0.23	0.85(0.5-1.3)		0.48	1.2(1.1-1.4)		0.001
Cumulative chewing (in years)[a]	0	181/1399	Reference		75/1399	Reference		48/1399	Reference		42/1399	Reference		346/1399	Reference	
	1-100	427/322	8.0(6.4-10.0)	<0.001	44/322	1.9(1.2-2.8)	0.004	32/322	2.2(1.3-3.6)	0.002	22/322	1.5(0.88-2.7)	0.12	525/322	5.2(4.3-6.3)	<0.001
	101-200	189/119	9.4(7.0-12.6)	<0.001	26/119	1.9(1.1-3.1)	0.01	18/119	2.2(1.1-3.9)	0.01	12/119	1.4(0.6-2.8)	0.35	246/119	5.5(4.2-7.1)	<0.001
	>200	147/78	11.2(8.0-15.7)	<0.001	21/78	2.1(1.1-3.7)	0.01	18/78	2.8(1.4-5.2)	0.001	8/78	1.2(0.50-2.6)	0.72	194/78	6.4(4.7-8.6)	<0.001
P_{trend}			<0.001			0.001			<0.001			0.38			<0.001	
Risk for every 10 chew years increase		1.01(1.01-1.02)		<0.001	1.01(1.0-1.02)		0.03	1.01(0.99-1.02)		0.08	0.98(0.96-1.0)		0.37	1.01(1.0-1.02)		0.001

Abbreviations: CI: Confidence Interval; OR: Odds Ratio

Ca/Co: Case/Control; All OR's are adjusted for age (continuous), gender, region and socio-economic status.

[a]number of times chewed per day X chewing duration of respective study participant.

Missing values are excluded from the analysis

Table 2.7. 2: Association of tobacco chewing by duration and frequency with sub-sites of head and neck cancer adjusted for age, gender, region and SES, tobacco smoking pack years and alcohol gram years.

Parameter	Categories	HNC Primary sub-site												All HNC cases(N=1320)		
		Oral cavity (N=950)			Oropharynx(N=166)			Hypopharynx(N=177)			Larynx(N=86)					
		Ca/Co	OR (95%CI)	p value	Ca/Co	OR (95%CI)	p value	Ca/Co	OR (95%CI)	p value	Ca/Co	OR (95%CI)	p value	Ca/Co	OR (95%CI)	p value
Chewing duration (in years)	0	181/1399	Reference		75/1399	Reference		48/1399	Reference		42/1399	Reference		346/1399	Reference	
	1-5	100/99	6.2(4.5-8.6)	<0.001	12/99	1.9(0.9-3.9)	0.07	8/99	1.8(0.8-4.3)	0.17	4/99	0.9(0.3-2.8)	0.88	124/99	4.5(3.3-6.0)	<0.001
	6-10	152/87	10.3(7.5-14.2)	<0.001	8/87	1.4(0.6-3.1)	0.38	6/87	1.7(0.7-4.2)	0.25	7/87	1.6(0.6-4.0)	0.28	173/87	6.9(5.1-9.3)	<0.001
	>10	515/337	9.1(7.3-11.5)	<0.001	71/337	2.2(1.4-3.2)	<0.001	55/337	2.8(1.8-4.5)	<0.001	32/337	1.5(0.9-2.6)	0.09	674/337	5.9(4.9-7.2)	<0.001
P_{trend}			<0.001			<0.001			<0.001			0.08			<0.001	
Number of times chewed per day	0	181/1399	Reference		75/1399	Reference		48/1399	Reference		42/1399	Reference		346/1399	Reference	
	1-10	658/476	8.3(6.7-10.2)	<0.001	80/476	1.9(1.3-2.9)	<0.001	61/476	2.4(1.5-3.7)	<0.001	42/476	1.5(0.9-2.5)	0.06	842/476	5.5(4.6-6.6)	<0.001
	11-20	96/38	14.4(9.4-22)	<0.001	9/38	2.3(0.9-5.6)	0.06	5/38	2.5(0.8-7.2)	0.09	0/38	NE	NE	110/38	8.7(5.8-13.2)	<0.001
	>20	10/7	7.2(2.6-20)	<0.001	2/7	3.2(0.5-19.3)	0.19	2/7	5.0(0.8-29.5)	0.07	1/7	3.2(0.3-29.8)	0.29	15/7	5.9(2.3-15.3)	<0.001
P_{trend}			<0.001			<0.001			<0.001			0.21			<0.001	
Risk for every 5 number of times chewed per day increase		1.2(1.1-1.4)		<0.001	1.1(0.93-1.4)		0.18	1.1(0.85-1.4)		0.40	0.83(0.52-1.3)		0.44	1.2(1.0-1.3)		0.001
Cumulative chewing (in years)[a]	0	181/1399	Reference		75/1399	Reference		48/1399	Reference		42/1399	Reference		346/1399	Reference	
	1-100	427/322	8.1(6.5-10.1)	<0.001	44/322	2.02(1.3-3.1)	0.001	32/322	2.2(1.3-3.7)	0.002	22/322	1.5(0.8-2.6)	0.15	525/322	5.5(4.5-6.7)	<0.001
	101-200	189/119	9.5(7-12.7)	<0.001	26/119	2.03(1.1-3.5)	0.01	18/119	2.4(1.3-4.6)	0.004	12/119	1.4(0.7-2.9)	0.29	246/119	5.9(4.5-7.7)	<0.001
	>200	147/78	10.8(7.7-15.3)	<0.001	21/78	2.07(1.1-3.8)	0.02	18/78	3.0(1.5-6)	0.001	8/78	1.2(0.5-2.9)	0.57	194/78	6.6(4.8-9)	<0.001
P_{trend}			<0.001			0.001			<0.001			0.27			<0.001	
Risk for every 10 chew years increase		1.01(1.0-1.02)		0.001	1.1(0.93-1.4)		0.11	1.01(0.99-1.0)		0.11	0.99(0.96-1.0)		0.56	1.01(1.0-1.02)		0.002

Abbreviations: CI: Confidence Interval; OR: Odds Ratio

Ca/Co: Case/Control; All OR's are adjusted for age (continuous), gender, region, socio-economic status, tobacco smoking pack years and alcohol gram years.

[a]number of times chewed per day X chewing duration of respective study participant.

Missing values are excluded from the analysis

Table 2.8. 1: Association of chewing duration and frequency of tobacco products with Oral cavity cancer adjusted for age, gender, region and SES.

Parameter	Categories	BQ+T[a]			Tobacco quid[b]			*Gutka*			*Khaini*		
		Ca/Co	OR (95% CI)	p value	Ca/Co	OR (95% CI)	p value	Ca/Co	OR (95% CI)	p value	Ca/Co	OR (95% CI)	p value
Chewing duration (in years)	0	181/1399	Reference		181/1399	Reference		181/1399	Reference		181/1399	Reference	
	1-5	21/15	8.7(4.4-17.5)	<0.001	43/25	10.2(6.0-17.4)	<0.001	52/24	15.1(8.8-25.6)	<0.001	7/7	6.5(2.2-19.2)	0.001
	6-10	28/11	15.9(7.7-32.9)	<0.001	70/20	20.7(12.1-35.3)	<0.001	82/19	31.1(17.9-53.9)	<0.001	18/7	16.3(6.6-40.3)	<0.001
	>10	132/61	12.2(8.6-17.5)	<0.001	255/81	18.1(13.2-24.6)	<0.001	170/29	38.4(24.6-59.8)	<0.001	68/35	10.7(6.8-16.8)	<0.001
P_{trend}			<0.001			<0.001			<0.001			<0.001	
Number of times chewed per day	0	181/1399	Reference		181/1399	Reference		181/1399	Reference		181/1399	Reference	
	1-10	165/79	12.2(8.8-16.8)	<0.001	326/114	16.6(12.5-21.9)	<0.001	262/66	27.0(19.2-38.0)	<0.001	83/45	10.7(7.0-16.1)	<0.001
	11-20	12/5	13.4(4.6-38.9)	<0.001	36/9	21.6(10.1-46.1)	<0.001	32/6	36.8(14.9-91.0)	<0.001	10/4	13.2(4.0-43.6)	<0.001
	>20	3/5	6.6(1.2-32.4)	0.02	3/2	11.6(1.9-71.0)	0.008	7/2	25.4(5.1-126.3)	<0.001	1/0	NE	NE
P_{trend}			<0.001			<0.001			<0.001			<0.001	
Risk for every 5 number of times chewed per day increase		1.05(0.78-1.4)		0.73	1.07(0.83-1.3)		0.59	1.2(0.92-1.5)		0.17	1.2(0.82-1.98)		0.27
Cumulative chewing(in years)[c]	0	181/1399	Reference		181/1399	Reference		181/1399	Reference		181/1399	Reference	
	1-100	96/49	12.8(8.4-18.2)	<0.001	209/73	17.2(12.4-23.7)	<0.001	208/61	23.7(16.6-33.8)	<0.001	54/30	11.3(6.9-18.6)	<0.001
	101-200	48/25	10.4(6.1-17.6)	<0.001	94/33	15.9(10.2-24.7)	<0.001	52/8	39.6(18.2-86.2)	<0.001	21/11	9.6(4.4-20.5)	<0.001
	>200	36/13	13.9(7.0-27.4)	<0.001	62/18	18.3(10.4-32.3)	<0.001	41/3	92.7(28.1-305.7)	<0.001	18/8	10.9(4.5-25.9)	<0.001
P_{trend}			<0.001			<0.001			<0.001			<0.001	

Abbreviations: CI: Confidence Interval; OR: Odds Ratio; NE: Not Estimable due to small number or no controls.

Ca/Co: Case/Control; All OR's are adjusted for age (continuous), gender, region and socio-economic status

[a]Betel leaf with tobacco and/or *areca nut and* or lime and/or *catachu*.

[b]Chewing tobacco with lime and/or *areca nut* and/or *catachu* without betel leaf.

[c]number of times chewed per day X chewing duration of respective study participant.

Missing values are excluded from the analysis

Table 2.8. 2: Association of chewing duration and frequency of tobacco products with Oral cavity cancer adjusted for age, gender, region, SES, tobacco smoking pack years and alcohol gram years.

Parameter	Categories	BQ+T[a]			Tobacco quid[b]			*Gutka*			*Khaini*		
		Ca/Co	OR (95% CI)	p value	Ca/Co	OR (95% CI)	p value	Ca/Co	OR (95% CI)	p value	Ca/Co	OR (95% CI)	p value
Chewing duration (in years)	0	181/1399	Reference		181/1399	Reference		181/1399	Reference		181/1399	Reference	
	1-5	21/15	8.9(4.4-18.0)	<0.001	43/25	10.3(6.0-17.5)	<0.001	52/24	15.5(9.1-26.4)	<0.001	7/7	6.4(2.1-19.2)	0.001
	6-10	28/11	16.1(7.8-33.3)	<0.001	70/20	20.9(12.3-35.8)	<0.001	82/19	31.5(18.2-54.6)	<0.001	18/7	16.8(6.8-41.67	<0.001
	>10	132/61	12.3(8.5-17.7)	<0.001	255/81	18.2(13.3-24.9)	<0.001	170/29	37.2(23.9-58.1)	<0.001	68/35	10.6(6.7-16.9)	<0.001
P_{trend}			<0.001			<0.001			<0.001			<0.001	
Number of times chewed per day	0	181/1399	Reference		181/1399	Reference		181/1399	Reference		181/1399	Reference	
	1-10	165/79	12.3(8.9-17.1)	<0.001	326/114	16.8(12.7-22.2)	<0.001	262/66	27.1(19.3-28.2)	<0.001	83/45	10.9(7.1-16.5)	<0.001
	11-20	12/5	11.9(4-35.2)	<0.001	36/9	20.8(9.6-44.6)	<0.001	32/6	34.9(14-86.5)	<0.001	10/4	11.7(3.4-39.1)	<0.001
	>20	3/5	6.2(1.2-31.7)	0.027	3/2	11.3(1.8-69)	0.008	7/2	21.9(4.3-109.7)	<0.001	1/0	NE	
P_{trend}			<0.001			<0.001			<0.001			<0.001	
Risk for every 5 number of times chewed per day increase		1.05(0.7-1.4)		0.71	1.09(0.8-1.4)		0.47	1.1(0.9-1.5)		0.20	1.2(0.78-1.9)		0.36
Cumulative chewing(in years)[c]	0	181/1399	Reference		181/1399	Reference		181/1399	Reference		181/1399	Reference	
	1-100	96/49	12.6(8.5-18.6)	<0.001	209/73	17.5(12.6-24.2)	<0.001	208/61	23.9(16.8-34.2)	<0.001	54/30	11.5(7-18.8)	<0.001
	101-200	48/25	10.3(6-17.5)	<0.001	94/33	15.9(10.2-24.8)	<0.001	52/8	39.2(18-85.4)	<0.001	21/11	10.3(4.7-22.2)	<0.001
	>200	36/13	13.8(6.9-27.2)	<0.001	62/18	18(10.2-31.9)	<0.001	41/3	84.7(25.6-280.2)	<0.001	18/8	9.8(4-23.7)	<0.001
P_{trend}			<0.001			<0.001			<0.001			<0.001	

Abbreviations: CI: Confidence Interval; OR: Odds Ratio; NE: Not Estimable due to small number or no controls.

Ca/Co: Case/Control; All OR's are adjusted for age (continuous), gender, region, socio-economic status, tobacco smoking pack years and alcohol gram years.

[a]Betel leaf with tobacco and/or *areca nut and/* or lime and/or *catachu.*

[b]Chewing tobacco with lime and/or *areca nut and* or *catachu* without betel leaf.

[c]number of times chewed per day X chewing duration of respective study participant.

Missing values are excluded from the analysis

Table 2.9. 1: Association of chewing duration and frequency of tobacco products with Oral cavity cancer adjusted for age, gender, region and SES.

Parameter	Categories	*Mawa*			*Mishri*			Other tobacco[a]			Other without tobacco[b]		
		Ca/Co	OR (95% CI)	p value	Ca/Co	OR (95% CI)	p value	Ca/Co	OR (95% CI)	p value	Ca/Co	OR (95% CI)	p value
Chewing duration (in years)	0	181/1399	Reference		181/1399	Reference		181/1399	Reference		181/1399	Reference	
	1-5	5/10	3.1(1.0-9.4)	0.04	4/8	3.5(1.0-12.0)	0.04	11/4	21.4(6.6-69.4)	<0.001	3/6	3.5(0.8-14.7)	0.08
	6-10	10/4	15.3(4.7-50.5)	<0.001	7/6	9.3(3.0-28.8)	<0.001	6/3	14.8(3.6-60.8)	<0.001	4/4	6.9(1.7-28.9)	0.007
	>10	33/26	7.2(4.1-12.4)	<0.001	24/24	5.8(3.1-10.8)	<0.001	22/5	28.4(10.9-77.1)	<0.001	14/11	9.0(3.9-20.6)	<0.001
P_{trend}		<0.001			<0.001			<0.001			<0.001		
Number of times chewed per day	0	181/1399	Reference		181/1399	Reference		181/1399	Reference		181/1399	Reference	
	1-10	45/35	7.7(4.7-12.4)	<0.001	33/38	5.6(3.3-9.3)	<0.001	38/11	24.2(12.0-49.1)	<0.001	17/21	5.8(2.9-11.4)	<0.001
	11-20	4/4	5.2(1.2-21.4)	0.02	1/0	NE		1/1	6.7(0.41-108.1)	0.18	4/0	NE	
	>20	No observations											
P_{trend}		<0.001			<0.001			<0.001			<0.001		
Risk for every 5 number of times chewed per day increase		0.88(0.52-1.4)		0.64	3.4(0.67-17.6)		0.14	3.1(0.62-15.3)		0.16	3.4(0.98-12.0)		0.05
Cumulative chewing(in years)[c]	0	181/1399	Reference		181/1399	Reference		181/1399	Reference		181/1399	Reference	
	1-100	33/21	9.8(5.4-17.5)	<0.001	26/33	5.1(2.9-8.9)	<0.001	29/10	20.6(9.6-43.9)	<0.001	11/17	4.7(2.1-10.4)	<0.001
	101-200	8/11	3.9(1.5-9.9)	0.005	6/5	7.9(2.2-26.6)	0.001	7/0	NE	NE	6/3	13.7(3.3-56.3)	<0.001
	>200	7/7	5.1(1.7-15.1)	0.003	2/0	NE		3/2	7.5(1.2-46.2)	0.03	4/1	23.2(2.5-211.5)	0.005
P_{trend}		<0.001			<0.001			<0.001			<0.001		

Abbreviations: CI: Confidence Interval; OR: Odds Ratio; NE: Not Estimable due to small number or no controls.

Ca/Co: Case/Control; All OR's are adjusted for age (continuous), gender, region and socio-economic status.

[a]Chewing or application of *gul and/or gudhakh and/or naash* and/or *zarda.*

[b]Chewing of *areca nut* and/or betel leaf and/or *Paan masala* without tobacco

[c]number of times chewed per day X chewing duration of respective study participant.

Missing values are excluded from the analysis

Table 2.9. 2: Association of chewing duration and frequency of tobacco products with Oral cavity cancer adjusted for age, gender, region, SES, tobacco smoking pack years and alcohol gram years.

Parameter	Categories	*Mawa*			*Mishri*			Other tobacco[a]			Others without tobacco[b]		
		Ca/Co	OR (95% CI)	p value	Ca/Co	OR (95% CI)	p value	Ca/Co	OR (95% CI)	p value	Ca/Co	OR (95% CI)	p value
Chewing duration (in years)	0	181/1399	Reference		181/1399	Reference		181/1399	Reference		181/1399	Reference	
	1-5	5/10	3.2(1.1-9.7)	0.03	4/8	2.9(0.7-10.7)	0.12	11/4	21.4(6.5-69.7)	<0.001	3/6	3.2(0.7-13.4)	0.11
	6-10	10/4	16.1(4.9-53.1)	<0.001	7/6	9.8(3.2-30.1)	<0.001	6/3	14.8(3.6-61.0)	<0.001	4/4	7.3(1.7-30.5)	0.006
	>10	33/26	7.1(4.0-12.3)	<0.001	24/24	5.9(3.2-11.0)	<0.001	22/5	26.9(9.8-73.4)	<0.001	14/11	7.8(3.3-18.2)	<0.001
P_{trend}		<0.001			<0.001			<0.001			<0.001		
Number of times chewed per day	0	181/1399	Reference		181/1399	Reference		181/1399	Reference		181/1399	Reference	
	1-10	45/35	7.6(4.7-12.5)	<0.001	33/38	5.6(3.3-9.4)	<0.001	38/11	23.5(11.6-47.8)	<0.001	17/21	5.3(2.7-10.7)	<0.001
	11-20	4/4	5.2(1.2-21.8)	0.02	1/0	NE		1/1	7.1(0.44-116)	0.16	4/0	NE	
	>20	No observations											
P_{trend}		<0.001			<0.001			<0.001			<0.001		
Risk for every 5 number of times chewed per day increase		0.8(0.5-1.5)		0.68	3.5(0.6-19.3)		0.15	3.9(0.6-23.3)		0.12	2.9(0.7-12.2)		0.12
Cumulative chewing(in years)[c]	0	181/1399	Reference		181/1399	Reference		181/1399	Reference		181/1399	Reference	
	1-100	33/21	9.7(5.3-17.4)	<0.001	26/33	5.12(2.9-9)	<0.001	29/10	20.1(9.4-43.1)	<0.001	11/17	4.6(2-10.3)	<0.001
	101-200	8/11	3.9(1.5-10.2)	0.004	6/5	7.6(2.2-26.3)	0.001	7/0	NE		6/3	11.9(2.7-50.7)	0.001
	>200	7/7	5.3(1.7-15.8)	0.003	2/0	NE		3/2	6.1(0.9-41.3)	0.06	4/1	15.1(1.5-147.8)	0.01
P_{trend}		<0.001			<0.001			<0.001			<0.001		

Abbreviations: CI: Confidence Interval; OR: Odds Ratio; NE: Not Estimable due to small number or no controls.

Ca/Co: Case/Control; All OR's are adjusted for age (continuous), gender, region, socio-economic status, tobacco smoking pack years and alcohol gram years.

[a]Chewing or application of *gul and/or gudhakhu and/or naash* and/or *zarda*.

[b]Chewing of *areca nut* and/or betel leaf and/or *Paan masala* without tobacco

[c]number of times chewed per day X chewing duration of respective study participant.

Missing values are excluded from the analysis

Table 2.10. 1: Risk estimates of tobacco products for HNC primary sub-sites adjusted for age, gender, region and SES.

Categories	HNC Primary sub-site												All HNC cases(N=1320)		
	Oral cavity (N=950)			Oropharynx(N=166)			Hypopharynx(N=177)			Larynx(N=86)					
	Ca/Co	OR[a] (95% CI)	p value	Ca/Co	OR[a] (95% CI)	p value	Ca/Co	OR[a] (95% CI)	p value	Ca/Co	OR[a] (95% CI)	p value	Ca/Co	OR[a] (95% CI)	p value
Never chewer	181/1399	Reference		75/1399	Reference		48/1399	Reference		42/1399	Reference		346/1399	Reference	
Tobacco products with areca nut[c]	575/228	15.1 (11.9-19.1)	<0.001	54/228	2.5 (1.6-3.7)	<0.001	39/228	2.8 (1.7-4.5)	<0.001	25/228	1.9 (1.1-3.4)	0.01	694/228	9.3 (7.5-11.3)	<0.001
Tobacco products without areca nut[d]	161/97	10.0 (7.4-13.5)	<0.001	19/97	2.3 (1.3-4.2)	0.005	20/97	3.8 (2.1-6.8)	<0.001	8/97	1.6 (0.7-3.7)	0.26	209/97	6.9 (5.2-9.1)	<0.001

Table 2.10. 2: Risk estimates of tobacco products for HNC primary sub-sites adjusted for age, gender, region, SES, tobacco smoking pack years and alcohol gram years.

Categories	HNC Primary sub-site												All HNC cases(N=1320)		
	Oral cavity (N=950)			Oropharynx(N=166)			Hypopharynx(N=177)			Larynx(N=86)					
	Ca/Co	OR[b] (95% CI)	p value	Ca/Co	OR[b] (95% CI)	p value	Ca/Co	OR[b] (95% CI)	p value	Ca/Co	OR[b] (95% CI)	p value	Ca/Co	OR[b] (95% CI)	p value
Never chewer	181/1399	Reference		75/1399	Reference		48/1399	Reference		42/1399	Reference		346/1399	Reference	
Tobacco products with areca nut	575/228	15.3 (12.1-19.3)	<0.001	54/228	2.7 (1.7-4.1)	<0.001	39/228	3.0 (1.8-5.0)	<0.001	25/228	1.9 (1.1-3.4)	0.02	694/228	9.9 (8.1-12.2)	<0.001
Tobacco products without areca nut	161/97	10.0 (7.3-13.6)	<0.001	19/97	2.7 (1.4-4.9)	0.001	20/97	4.5 (2.4-8.3)	<0.001	8/97	1.7 (0.7-4.0)	0.20	209/97	7.3 (5.4-9.7)	<0.001

Abbreviations: CI: Confidence Interval; OR: Odds Ratio; Ca/Co: Case/Control

[a]OR's are adjusted for age (continuous), gender, region and socio-economic status.

[b]OR's are adjusted for age (continuous), gender, region, socio-economic status, tobacco smoking pack years and alcohol gram years.

[c]Includes tobacco products *betel leaf with tobacco and/or tobacco quid and/or Gutka and/or Mawa.*; [d]Includes tobacco products *Khaini and/or zarda and/or gul and/or gudhakhu and/or Mishri and/or creamy snuff.* The classification of tobacco products has been adapted from WHO report on Smokeless use and Public Health in India[120].

Table 2.11. 1: Risk estimates of tobacco products by preparation method for HNC primary sub-sites adjusted for age, gender, region, SES, tobacco smoking pack years and alcohol gram years.

Categories	HNC Primary sub-site												All HNC cases(N=1320)		
	Oral cavity (N=950)			Oropharynx(N=166)			Hypopharynx(N=177)			Larynx(N=86)					
	Ca/Co	OR[a] (95%CI)	p value	Ca/Co	OR[a] (95%CI)	p value	Ca/Co	OR[a] (95%CI)	p value	Ca/Co	OR[a] (95%CI)	p value	Ca/Co	OR[a] (95%CI)	p value
Never chewer	181/1399	Reference		75/1399	Reference		48/1399	Reference		42/1399	Reference		346/1399	Reference	
Commercially prepared[c]	336/83	27.3 (19.9-37.3)	<0.001	20/83	4.8 (2.6-9.0)	<0.001	8/83	2.6 (1.1-6.0)	0.02	7/83	2.6 (1.1-6.5)	0.03	372/83	18.0 (13.3-24.2)	<0.001
Manually prepared[d]	443/241	10.6 (8.4-13.3)	<0.001	52/241	2.2 (1.4-3.3)	<0.001	45/241	2.9 (1.9-4.7)	<0.001	25/241	1.8 (1.0-3.0)	0.05	565/241	6.7 (5.4-8.1)	<0.001

Table 2.11. 2: Risk estimates of tobacco products by preparation method for HNC primary sub-sites adjusted for age, gender, region, SES, tobacco smoking pack years and alcohol gram years.

Categories	HNC Primary sub-site												All HNC cases(N=1320)		
	Oral cavity (N=950)			Oropharynx(N=166)			Hypopharynx(N=177)			Larynx(N=86)					
	Ca/Co	OR[b] (95%CI)	p value	Ca/Co	OR[b] (95%CI)	p value	Ca/Co	OR[b] (95%CI)	p value	Ca/Co	OR[b] (95%CI)	p value	Ca/Co	OR[b] (95%CI)	p value
Never chewer	181/1399	Reference		75/1399	Reference		48/1399	Reference		42/1399	Reference		346/1399	Reference	
Commercially prepared[c]	336/83	27.1 (19.8-37.1)	<0.001	20/83	4.6 (2.4-8.7)	<0.001	8/83	2.7 (1.1-6.4)	0.03	7/83	2.5 (0.98-6.4)	0.05	372/83	18.4 (13.6-24.8)	<0.001
Manually prepared[d]	443/241	10.7 (8.5-13.6)	<0.001	52/241	2.4 (1.6-3.8)	<0.001	45/241	3.3 (2.0-5.4)	<0.001	25/241	1.8 (1.0-3.2)	0.04	565/241	7.3 (5.9-8.9)	<0.001

Abbreviations: CI: Confidence Interval; OR: Odds Ratio; Ca/Co: Case/Control

[a]OR's are adjusted for age (continuous), gender, region and socio-economic status.

[b]OR's are adjusted for age (continuous), gender, region, socio-economic status, tobacco smoking pack years and alcohol gram years.

[c]Includes *Gutka and/or gul and/or gudhakhu and/or naash and/or zarda.*

[d]Includes BQ+T and/or tobacco quid and/or *Khaini* and/or *Mawa* and/or *Mishri.*

Table 2.12. 1: Association of alcohol drinking with sub-sites of head and neck cancer adjusted for age, gender, region and SES.

Parameter	Categories	HNC Primary sub-site												All HNC cases(N=1320)		
		Oral cavity (N=950)			Oropharynx(N=166)			Hypopharynx(N=177)			Larynx(N=86)					
		Ca/Co	OR (95%CI)	p value	Ca/Co	OR (95%CI)	p value	Ca/Co	OR (95%CI)	p value	Ca/Co	OR (95%CI)	p value	Ca/Co	OR (95%CI)	p value
Alcohol drinking	Never	695/1721	Reference		112/1721	Reference		87/1721	Reference		70/1721	Reference		965/1721	Reference	
	Ever	255/203	2.1 (1.7-2.6)	<0.001	54/203	2.3 (1.5-3.4)	<0.001	30/203	1.68 (1.05-2.6)	0.03	16/203	0.91 (0.50-1.6)	0.76	355/203	2.05 (1.6-2.5)	<0.001
Type of alcohol drinking	Beer	64/51	2.5 (1.6-3.6)	<0.001	7/51	1.7 (0.7-3.9)	0.23	6/51	1.9 (0.7-4.7)	0.18	4/51	1.3 (0.4-3.9)	0.61	81/51	2.3 (1.5-3.3)	<0.001
	Whisky	134/107	2.3 (1.7-3.0)	<0.001	22/107	2.3 (1.3-4.0)	0.001	16/107	2.2 (1.2-3.9)	0.01	7/107	1.0 (0.44-2.3)	0.97	179/107	2.2 (1.7-2.9)	<0.001
	Country Spirit	98/54	2.4 (1.7-3.5)	<0.001	29/54	3.1 (1.8-5.2)	<0.001	16/54	2.1 (1.1-4.0)	0.02	9/54	1.2 (0.5-2.6)	0.67	152/54	2.6 (1.8-3.5)	<0.001
	Toddy	13/10	1.6 (0.6-3.8)	0.25	2/10	1.1 (0.2-5.4)	0.90	1/10	0.76 (0.09-6.3)	0.80	1/10	0.70 (0.08-6.0)	0.74	17/10	1.4 (0.6-3.1)	0.42
	Others[a]	23/34	1.5 (0.8-2.5)	0.16	3/34	0.84 (0.2-2.9)	0.79	0/34	NE		2/34	0.88 (0.2-3.8)	0.87	28/34	1.2 (0.72-2.0)	0.44

Abbreviations: CI: Confidence Interval; OR: Odds Ratio; NE: Not Estimable due to small number or no cases/controls.
Ca/Co: Case/Control; All OR's are adjusted for age (continuous), gender, region and socio-economic status.
[a]Consumption of vodka and/or rum and/or gin and/or wine and/or brandy.
Missing values are excluded from the analysis.

Table 2.12. 2: Association of alcohol drinking with sub-sites of head and neck cancer adjusted for age, gender, region, SES, tobacco chewing and tobacco smoking pack years.

Parameter	Categories	HNC Primary sub-site												All HNC cases(N=1320)		
		Oral cavity (N=950)			Oropharynx(N=166)			Hypopharynx(N=177)			Larynx(N=86)					
		Ca/Co	OR (95%CI)	p value	Ca/Co	OR (95%CI)	p value	Ca/Co	OR (95%CI)	p value	Ca/Co	OR (95%CI)	p value	Ca/Co	OR (95%CI)	p value
Alcohol drinking	Never	695/1721	Reference		112/1721	Reference		87/1721	Reference		70/1721	Reference		965/1721	Reference	
	Ever	255/203	1.39(1.1-1.7)	0.006	54/203	1.8(1.2-2.8)	0.002	30/203	1.2(0.7-2.1)	0.305	16/203	0.8(0.4-1.5)	0.524	355/203	1.3(1-1.6)	0.007
Type of alcohol drinking	Beer	64/51	1.6(1-2.55)	0.02	7/51	1.4(0.5-3.4)	0.42	6/51	1.3(0.5-3.5)	0.54	4/51	1.2(0.4-3.6)	0.72	81/51	1.5(1.0-2.2)	0.04
	Whisky	134/107	1.4(1.0-2.0)	0.01	22/107	1.8(1.0-3.2)	0.02	16/107	1.7(0.9-3.2)	0.08	7/107	0.9(0.4-2.1)	0.86	179/107	1.4(1.0-1.8)	0.01
	Country Spirit	98/54	1.6(1.1-2.4)	0.01	29/54	2.5(1.4-4.4)	0.001	16/54	1.5(0.7-3.0)	0.18	9/54	1.0(0.4-2.3)	0.94	152/54	1.6(1.1-2.3)	0.005
	Toddy	13/10	1(0.4-2.4)	0.99	2/10	1.1(0.2-5.7)	0.85	1/10	0.6(0.07-5.8)	0.72	1/10	0.4(0.04-4.8)	0.52	17/10	0.9(0.3-2.1)	0.81
	Others[a]	23/34	0.9(0.5-1.7)	0.92	3/34	0.5(0.1-2.0)	0.33	0/34	NE		2/34	0.7(0.1-3.4)	0.73	28/34	0.7(0.4-1.3)	0.36

Abbreviations: CI: Confidence Interval; OR: Odds Ratio; NE: Not Estimable due to small number or no cases/controls.

Ca/Co: Case/Control; All OR's are adjusted for age (continuous), gender, region, socio-economic status, tobacco chewing and tobacco smoking pack years

[a]Consumption of vodka and/or rum and/or gin and/or wine and/or brandy.

Missing values are excluded from the analysis.

Table 2.13. 1: Association of alcohol drinking by duration and frequency with sub-sites of head and neck cancer adjusted for age, gender, region and SES.

Parameter	Categories	HNC Primary sub-site												All HNC cases(N=1320)		
		Oral cavity (N=950)			Oropharynx(N=166)			Hypopharynx(N=177)			Larynx(N=86)					
		Ca/Co	OR (95% CI)	p value	Ca/Co	OR (95% CI)	p value	Ca/Co	OR (95% CI)	p value	Ca/Co	OR (95% CI)	p value	Ca/Co	OR (95% CI)	p value
Drinking duration (in years)	0	695/1721	Reference		112/1721	Reference		87/1721	Reference		70/1721	Reference		965/1721	Reference	
	1-10	75/61	2.1 (1.5-3.0)	<0.001	6/61	1.4 (0.6-3.4)	0.46	6/61	1.8 (0.7-4.6)	0.19	2/61	0.66 (0.2-2.9)	0.58	89/61	1.9 (1.4-2.8)	<0.001
	11-20	92/60	2.5 (1.7-3.5)	<0.001	14/60	2.2 (1.1-4.2)	0.02	6/60	1.9 (0.5-3.1)	0.59	3/60	0.70 (0.2-2.3)	0.57	115/60	2.3 (1.6-3.2)	<0.001
	>30	87/82	1.8 (1.3-2.5)	0.001	34/82	2.8 (1.7-4.5)	<0.001	17/82	1.7 (0.9-3.1)	0.07	11/82	1.1 (0.5-2.2)	0.81	149/82	1.9 (1.4-2.5)	<0.001
P_{trend}			<0.001			<0.001			0.06			0.96			<0.001	
Risk for every 5 years increase in drinking			1.1(1.1-1.2)	<0.001		1.2(1.1-1.3)	<0.001		1.1(0.9-1.1)	0.08		1.03(0.9-1.1)	0.46		1.1(1.1-1.2)	<0.001
Cumulative drinking (in ethanol gram years)[a]	0	695/1721	Reference		112/1721	Reference		87/1721	Reference		70/1721	Reference		965/1721	Reference	
	Q1(296.2,316.3,261.2)[b]	41/44	1.5 (0.9-2.4)	0.06	1/44	0.26 (0.03-1.9)	0.19	2/44	0.74 (0.2-3.2)	0.69	0/44	NE		44/44	1.3 (0.8-1.9)	0.28
	Q2(1652.9,1537.2,117.2)	99/73	2.4 (1.7-3.3)	<0.001	15/73	2.0 (1.1-3.8)	0.03	5/73	0.77 (0.3-2.0)	0.59	1/73	0.16 (0.02-1.2)	0.08	120/73	2.1 (1.5-2.8)	<0.001
	Q3(6081.5,5565.8,3287.2)	63/53	2.0 (1.3-2.9)	<0.001	13/53	1.9 (0.96-3.6)	0.06	8/53	1.5 (0.7-3.4)	0.28	7/53	1.4 (0.6-3.2)	0.44	91/53	1.9 (1.4-2.8)	<0.001
	Q4(29165.2,16480.8,19513.7)	45/27	2.5 (1.5-4.2)	<0.001	23/27	6.1 (3.2-11.4)	<0.001	14/27	5.0 (2.4-10.4)	<0.001	6/27	2.2 (0.8-5.7)	0.12	88/27	3.4 (2.1-5.3)	<0.001
P_{trend}			<0.001			<0.001			0.001			0.49			<0.001	

Abbreviations: CI: Confidence Interval; OR: Odds Ratio; NE: Not Estimable due to small number or no cases/controls.

Ca/Co: Case/Control; All OR's are adjusted for age (continuous), gender, region and socio-economic status.

[a]Ethanol gram years were calculated considering that one liter of alcohol weighs 798gm of ethanol and that beer contains 5% ethanol in volume; whisky, country liquor, vodka and gin 41%; toddy 5% and wine 12%. After conversion to ethanol consumption in grams the cumulative was calculated by multiplying with drinking duration (in years) of each study participant.

[b]Quartiles of ethanol gram years based on study controls consumption. The values in the parenthesis are mean, median and inter-quartile range respectively.

Missing values are excluded from analysis.

Table 2.13. 2: Association of alcohol drinking by duration and frequency with sub-sites of head and neck cancer adjusted for age, gender, region, SES, tobacco chewing and tobacco smoking pack years.

Parameter	Categories	HNC Primary sub-site												All HNC cases(N=1320)		
		Oral cavity (N=950)			Oropharynx(N=166)			Hypopharynx(N=177)			Larynx(N=86)					
		Ca/Co	OR (95%CI)	p value	Ca/Co	OR (95%CI)	p value	Ca/Co	OR (95%CI)	p value	Ca/Co	OR (95%CI)	p value	Ca/Co	OR (95%CI)	p value
Drinking duration (in years)	0	695/1721	Reference		112/1721	Reference		87/1721	Reference		70/1721	Reference		965/1721	Reference	
	1-10	75/61	1.3 (0.8-1.9)	0.17	6/61	1.1 (0.4-2.9)	0.72	6/61	1.3 (0.5-3.4)	0.53	2/61	0.6 (0.1-2.6)	0.49	89/61	1.2 (0.8-1.8)	0.21
	11-20	92/60	1.6 (1.1-2.4)	0.006	14/60	1.8 (0.9-3.5)	0.08	6/60	1.0 (0.4-2.7)	0.87	3/60	0.6 (0.2-2.3)	0.54	115/60	1.5 (1.0-2.1)	0.02
	>30	87/82	1.2 (0.8-1.7)	0.27	34/82	2.2 (1.3-3.6)	0.002	17/82	1.29 (0.6-2.4)	0.41	11/82	0.9 (0.4-1.9)	0.89	149/82	1.2 (0.9-1.7)	0.13
P_{trend}			0.01			0.001			0.41			0.70			0.01	
Risk for every 5 years increase in drinking			1.06(1.0-1.1)	0.03		1.1(1.0-1.2)	0.002		1.03(0.9-1.1)	0.43		1.01(0.9-1.1)	0.72		1.06(1.0-1.1)	0.02
Cumulative drinking (in ethanol gram years)[a]	0	695/1721	Reference		112/1721	Reference		87/1721	Reference		70/1721	Reference		965/1721	Reference	
	Q1(296.2,316.3,261.2)[b]	41/44	0.9(0.6-1.6)	0.96	1/44	0.2(0.03-1.8)	0.16	2/44	0.6(0.1-2.9)	0.59	0/44	NE		44/44	0.8(0.5-1.3)	0.50
	Q2(1652.9,1537.2,117.2)	99/73	1.5(1.0-2.2)	0.01	15/73	1.6(0.8-3.2)	0.11	5/73	0.6(0.2-1.7)	0.40	1/73	0.1(.02-1.1)	0.06	120/73	1.4(1-1.9)	0.04
	Q3(6081.5,5565.8,3287.2)	63/53	1.3(0.8-2.1)	0.14	13/53	1.4(0.7-2.9)	0.27	8/53	1.0(0.4-2.3)	0.95	7/53	1.2(0.5-2.9)	0.59	91/53	1.2(0.8-1.8)	0.26
	Q4(29165.2,16480.8,19513.7)	45/27	1.7(0.9-2.9)	0.05	23/27	4.7(2.4-9)	<0.001	14/27	3.5(1.6-7.6)	0.001	6/27	1.7(0.6-4.8)	0.25	88/27	2.1(1.3-3.5)	0.002
P_{trend}			0.003			<0.001			0.03			0.76			0.001	

Abbreviations: CI: Confidence Interval; OR: Odds Ratio; NE: Not Estimable due to small number or no cases/controls.

Ca/Co: Case/Control; All OR's are adjusted for age (continuous), gender, region, socio-economic status, tobacco smoking pack years tobacco chewing.

[a]Ethanol gram years were calculated considering that one liter of alcohol weighs 798gm of ethanol and that beer contains 5% ethanol in volume; whisky, country liquor, vodka and gin 41%; toddy 5% and wine 12%. After conversion to ethanol consumption in grams the cumulative was calculated by multiplying with duration of duration (in years) of each study participant.

[b]Quartiles of ethanol gram years based on study controls consumption. The values in the parenthesis are mean, median and inter-quartile range respectively.

Missing values are excluded from analysis.

Table 2.14. 1: Association of alcohol drinking gram years with sub-sites of head and neck cancer adjusted for age, gender, region and SES.

Parameter	Categories	HNC Primary sub-site												All HNC cases(N=1320)		
		Oral cavity (N=950)			Oropharynx(N=166)			Hypopharynx(N=177)			Larynx(N=86)					
		Ca/Co	OR (95%CI)	p value	Ca/Co	OR (95%CI)	p value	Ca/Co	OR (95%CI)	p value	Ca/Co	OR (95%CI)	p value	Ca/Co	OR (95%CI)	p value
Beer consumption in gram years[a]	1-1708.2	54/43	2.5(1.6-3.8)	<0.001	4/43	1.2(0.4-3.5)	0.75	2/43	0.72(0.2-3.1)	0.66	4/43	1.6(0.5-4.8)	0.40	64/43	2.2(1.4-3.3)	<0.001
	>1708.2	9/7	2.5(0.9-6.8)	0.07	1/7	1.5(0.2-12.9)	0.71	3/7	5.9(1.4-24.5)	0.01	0/7	NE		13/7	2.5(0.97-6.4)	0.05
P_{trend}			<0.001			0.64			0.09			0.83			<0.001	
Whisky consumption in gram years	1-14322.6	120/103	2.2(1.6-2.9)	<0.001	19/103	2.1(1.2-3.7)	0.009	13/103	1.8(0.95-3.4)	0.07	6/103	0.9(0.4-2.1)	0.80	158/103	2.1(1.5-2.7)	<0.001
	>14322.6	10/1	14.2	0.01	2/1	16.6(1.3-203.1)	0.02	3/1	31.2(2.9-328.6)	0.004	1/1	NE		16/1	18.1(2.3-139.5)	0.005
P_{trend}			<0.001			0.001			0.001			0.70			<0.001	
Country spirit consumption in gram years	1-14322.6	73/42	2.3(1.5-3.5)	<0.001	19/42	2.6(1.4-4.8)	0.002	10/42	1.8(0.8-3.8)	0.13	5/42	0.94(0.3-2.5)	0.90	107/42	2.4(1.6-3.4)	<0.001
	>14322.6	22/10	2.9(1.3-6.4)	0.005	10/10	5.8(2.2-15.1)	<0.001	5/10	3.2(0.9-10.4)	0.05	2/10	1.4(0.2-7.1)	0.68	39/10	3.4(1.6-6.9)	0.001
P_{trend}			<0.001			<0.001			0.02			0.82			<0.001	
Toddy consumption in gram years	1-1195.74	9/3	3.5(0.9-13.2)	0.07	0/3	NE		1/3	3.4(0.3-37.4)	0.33	0/3	NE		10/3	2.6(0.7-9.8)	0.15
	>1195.74	4/6	0.9(0.2-3.2)	0.83	2/6	1.7(0.3-9.2)	0.54	0/6	NE		1/6	1.0(0.1-9.5)	0.98	7/6	0.9(0.3-2.9)	0.92
P_{trend}			0.45			0.67			0.58			0.89			0.56	
Other alcohol consumption in gram years	1-14322.6	17/31	1.3(0.7-2.3)	0.45	1/31	0.4(0.05-2.9)	0.35	0/31	NE		2/31	0.98(0.2-4.3)	0.97	20/31	1.1(0.6-1.9)	0.84
	>14322.6	1/0	NE		1/0	NE		0/0			0/0	NE		2/0	NE	
P_{trend}			0.26			0.80			NE			0.97			0.57	

Abbreviations: CI: Confidence Interval; OR: Odds Ratio; NE: Not Estimable due to small number or no cases/controls.

Ca/Co: Case/Control; All OR's are adjusted for age (continuous), gender, region and socio-economic status

[a]Ethanol gram years were calculated considering that one liter of alcohol weighs 798gm of ethanol and that beer contains 5% ethanol in volume; whisky, country liquor, vodka and gin 41%; toddy 5% and wine 12%. After conversion to ethanol consumption in grams the cumulative was calculated by multiplying with duration of duration (in years) of each study participant. Missing values are excluded from analysis.

Table 2.14. 2: Association of alcohol drinking gram years with sub-sites of head and neck cancer adjusted for age, gender, region, SES, tobacco smoking pack years and tobacco chewing.

Parameter	Categories	HNC Primary sub-site												All HNC cases(N=1320)		
		Oral cavity (N=950)			Oropharynx(N=166)			Hypopharynx(N=177)			Larynx(N=86)					
		Ca/Co	OR (95%CI)	p value	Ca/Co	OR (95%CI)	p value	Ca/Co	OR (95%CI)	p value	Ca/Co	OR (95%CI)	p value	Ca/Co	OR (95%CI)	p value
Beer consumption in gram years	1-1708.2	54/43	1.7(1.0-2.7)	0.02	4/43	1.03(0.3-3.1)	0.94	2/43	0.4(0.1-2.3)	0.36	4/43	1.5(0.4-4.6)	0.47	64/43	1.5(0.98-2.4)	0.05
	>1708.2	9/7	1.4(0.4-4.2)	0.51	1/7	1.1(0.1-10)	0.89	3/7	3.8(0.8-16.5)	0.06	0/7	NE		13/7	1.2(0.4-3.4)	0.65
P_{trend}			0.03			0.89			0.35			0.96			0.08	
Whisky consumption in gram years	1-14322.6	120/103	1.3(1.0-1.9)	0.04	19/103	1.6(0.93-2.9)	0.08	13/103	1.4(0.73-2.7)	0.29	6/103	0.8(0.3-1.9)	0.65	158/103	1.3(0.99-1.7)	0.05
	>14322.6	10/1	8.9(1.0-77.9)	0.04	2/1	11.5(0.99-135.5)	0.05	3/1	20.7(2.0-213.9)	0.01	1/1	8.2(0.4-140.8)	0.14	16/1	10.3(1.3-81.1)	0.02
P_{trend}			0.006			0.01			0.01			0.86			0.004	
Country spirit consumption in gram years	1-14322.6	73/42	1.5(0.98-2.3)	0.05	19/42	2.05(1.0-3.9)	0.02	10/42	1.2(0.5-2.7)	0.58	5/42	0.7(0.2-2.1)	0.58	107/42	1.4(0.9-2.1)	0.06
	>14322.6	22/10	2.3(1.0-5.5)	0.04	10/10	5.1(1.97-13.2)	0.001	5/10	2.6(0.7-9.0)	0.12	2/10	1.4(0.2-7.3)	0.63	39/10	2.4(1.1-5.2)	0.01
P_{trend}			0.007			<0.001			0.13			0.98			0.003	
Toddy consumption in gram years	1-1195.74	9/3	1.6(0.4-6.3)	0.44	0/3	NE		1/3	1.9(0.1-23.4)	0.61	0/3	NE		10/3	1.2(0.3-4.5)	0.76
	>1195.74	4/6	0.6(0.1-2.70	0.60	2/6	2.2(0.4-12.1)	0.34	0/6	NE		1/6	0.7(0.05-9.0)	0.81	7/6	0.8(0.2-2.9)	0.81
P_{trend}			0.94			0.53			0.60			0.66			0.93	
Other alcohol consumption in gram years	1-14322.6	17/31	0.7(0.4-1.5)	0.47	1/31	0.2(0.03-2.2)	0.23	0/31	NE		2/31	0.84(0.1-3.7)	0.82	20/31	0.6(0.3-1.2)	0.17
	>14322.6	1/0	NE		1/0	NE		0/0			0/0	NE		2/0	NE	
P_{trend}			0.86			0.37			NE			0.82			0.31	

Abbreviations: CI: Confidence Interval; OR: Odds Ratio; NE: Not Estimable due to small number or no cases/controls.

Ca/Co: Case/Control; All OR's are adjusted for age (continuous), gender, region, socio-economic status, tobacco chewing and alcohol gram years.

Ethanol gram years were calculated considering that one liter of alcohol weighs 798gm of ethanol and that beer contains 5% ethanol in volume; whisky, country liquor, vodka and gin 41%; toddy 5% and wine 12%. After conversion to ethanol consumption in grams the cumulative was calculated by multiplying with duration of duration (in years) of each study participant.

Missing values are excluded from analysis.

Table 2.15. 1: Joint association of tobacco smoking and alcohol drinking on oral cavity and overall head and neck cancer.

Parameter	Oral cavity (N=950)			All HNC cases(N=1320)		
	Ca/Co	**OR (95%CI)**	**$P_{interaction}$**	**Ca/Co**	**OR (95%CI)**	**$P_{interaction}$**
Ever smoker	302/357	1.44(1.1-1.8)	<0.001	547/357	2.3(1.9-2.8)	<0.001
Ever drinker	255/203	1.39(1.1-1.7)	0.006	355/203	1.3(1-1.6)	0.007
Ever smoker and drinker	132/102	1.9(1.4-2.7)	<0.001	217/102	2.8(2.1-3.8)	<0.001
Joint association of smoking and drinking						
Never smoker and never drinker	525/1466	Reference		635/1466	Reference	
level 1 drinker[a] and level 1 smoker[b]	7/5	1.8(0.46-7.0)	0.34	7/5	1.8(0.48-6.4)	0.34
level 1 drinker and level 2 smoker[b]	8/11	0.96(0.34-2.6)	0.59	9/11	1.0(0.38-2.6)	0.46
level 1 drinker and level 3 smoker[b]	4/6	1.0(0.24-4.2)	0.27	6/6	1.1(0.32-3.9)	0.07
level 1 drinker and level 4 smoker[b]	2/2	1.08(0.1-10.5)	0.90	2/2	0.8(0.08-7.4)	0.24
level 2 drinker[a] and level 1 smoker	9/6	2.1(0.64-6.6)	0.53	9/6	1.9(0.62-6.0)	0.58
level 2 drinker and level 2 smoker	12/8	1.9(0.71-5.1)	0.79	14/8	2.1(0.8-5.2)	0.72
level 2 drinker and level 3 smoker	19/11	2.5(1.1-5.9)	0.39	27/11	3.1(1.4-6.8)	0.12
level 2 drinker and level 4 smoker	4/8	0.86(0.22-3.3)	0.27	14/8	2.9(1.1-7.8)	0.34
level 3 drinker[a] and level 1 smoker	6/2	4.8(0.76-30.3)	0.17	6/2	3.4(0.62-19.5)	0.31
level 3 drinker and level 2 smoker	3/10	0.29(0.07-1.1)	0.01	5/10	0.42(0.13-1.33)	0.009
level 3 drinker and level 3 smoker	8/8	1.5(0.48-4.4)	0.19	**13/8**	**2.0(0.73-5.4)**	**0.05***
level 3 drinker and level 4 smoker	13/10	4.0(1.5-10.6)	0.20	25/10	5.6(2.4-12.7)	0.85
level 4 drinker[a] and level 1 smoker	4/2	1.5(0.27-8.6)	0.56	7/2	2.9(0.57-15.1)	0.28
level 4 drinker and level 2 smoker	6/2	2.2(0.42-11.0)	0.66	6/2	2.0(0.39-10.1)	0.97
level 4 drinker and level 3 smoker	7/5	3.3(0.86-13.2)	0.75	21/5	6.2(2.1-17.8)	0.84
level 4 drinker and level 4 smoker	**12/3**	**8.2(1.9-35)**	**0.03**	**32/3**	**18.1(5.2-62.8)**	**0.04***

Abbreviations: CI: Confidence Interval; OR: Odds Ratio

Ca/Co: Case/Control; All OR's are adjusted for age (continuous), gender, region, socio-economic status and tobacco chewing.

[a]Cumulative alcohol consumption: level 1 drinker- ≤548.1 gram years; level 2 drinker- >548.1- ≤ 3380.31gram years; level 3 drinker- >3380.31- ≤9871.313 gram years; level 4 drinker- >9871.313 gram years.

[b]Cumulative smoking exposure: level 1 smoker- ≤24 pack years; level 2 smoker- >24- ≤66 pack years; level 3 smoker- >66- ≤216 pack years; level 4 smoker: >216 pack years.

*Multiplicative interaction significant at α=0.05

Table 2.15. 2: Joint association risk estimates of heavy alcohol drinkers at various smoker levels

Smoker levels	Drinker level	Oral cavity (N=950)			All HNC cases(N=1320)		
		Ca/Co	**OR (95%CI)**	**$P_{interaction}$**	**Ca/Co**	**OR (95%CI)**	**$P_{interaction}$**
Never drinker and never smoker		525/1466	Reference		635/1466	Reference	
1	4	4/2	1.5(0.27-8.6)	0.56	7/2	2.9(0.57-15.1)	0.28
2	4	6/2	2.2(0.42-11.0)	0.66	6/2	2.0(0.39-10.1)	0.97
3	4	7/5	3.3(0.86-13.2)	0.75	21/5	6.2(2.1-17.8)	0.84
4	**4**	**12/3**	**8.2(1.9-35)**	**0.03***	**32/3**	**18.1(5.2-62.8)**	**0.04***

Abbreviations: CI: Confidence Interval; OR: Odds Ratio

Ca/Co: Case/Control; All OR's are adjusted for age (continuous), gender, region, socio-economic status and tobacco chewing.

[a]Cumulative alcohol consumption: level 1 drinker- ≤548.1 gram years; level 2 drinker- >548.1- ≤ 3380.31gram years; level 3 drinker- >3380.31- ≤9871.313 gram years; level 4 drinker- >9871.313 gram years.

[b]Cumulative smoking exposure: level 1 smoker- ≤24 pack years; level 2 smoker- >24- ≤66 pack years; level 3 smoker- >66- ≤216 pack years; level 4 smoker: >216 pack years.

*Multiplicative interaction significant at α=0.05

Table 2.15. 3: Joint association risk estimates of heavy smokers at various drinker levels

Drinker levels	Smoker level	Oral cavity (N=950)			All HNC cases(N=1320)		
		Ca/Co	**OR (95%CI)**	**$P_{interaction}$**	**Ca/Co**	**OR (95%CI)**	**$P_{interaction}$**
Never drinker and never smoker		525/1466	Reference		635/1466	Reference	
1	4	2/2	1.08(0.1-10.5)	0.90	2/2	0.8(0.08-7.4)	0.24
2	4	4/8	0.86(0.22-3.3)	0.27	14/8	2.9(1.1-7.8)	0.34
3	4	13/10	4.0(1.5-10.6)	0.20	25/10	5.6(2.4-12.7)	0.85
4	**4**	**12/3**	**8.2(1.9-35)**	**0.03***	**32/3**	**18.1(5.2-62.8)**	**0.04***

Abbreviations: CI: Confidence Interval; OR: Odds Ratio

Ca/Co: Case/Control; All OR's are adjusted for age (continuous), gender, region, socio-economic status and tobacco chewing.

[a]Cumulative alcohol consumption: level 1 drinker- ≤548.1 gram years; level 2 drinker- >548.1- ≤ 3380.31gram years; level 3 drinker- >3380.31- ≤9871.313 gram years; level 4 drinker- >9871.313 gram years.

[b]Cumulative smoking exposure: level 1 smoker- ≤24 pack years; level 2 smoker- >24- ≤66 pack years; level 3 smoker- >66- ≤216 pack years; level 4 smoker: >216 pack years.

*Multiplicative interaction significant at α=0.05

Table 2.15. 4: Joint association of tobacco chewing and alcohol drinking on oral cavity and overall head and neck cancer.

Parameter	Oral cavity (N=950)			All HNC cases(N=1320)		
	Ca/Co	**OR (95%CI)**	**$P_{interaction}$**	**Ca/Co**	**OR (95%CI)**	**$P_{interaction}$**
Ever chewer	779/525	8.7(7.1-10.7)	<0.001	974/525	5.8(4.8-6.9)	<0.001
Ever drinker	255/203	1.39(1.1-1.7)	0.006	355/203	1.3(1-1.6)	0.007
Ever chewer and drinker	228/125	3.1(2.4-3.9)	<0.001	300/125	2.8(2.2-3.6)	<0.001
Joint association of chewing and drinking						
Never chewer and never drinker	154/1321	Reference		291/1321	Reference	
level 1 drinker[a] and level 1 chewer[b]	27/16	9.9(5.1-19.1)	0.6	28/16	6.0(3.1-11.4)	0.23
level 1 drinker and level 2 chewer[b]	8/3	16.1(4.1-62.0)	0.41	9/3	9.1(2.4-34.2)	0.16
level 1 drinker and level 3 chewer[b]	4/6	4.1(1.1-15.1)	0.39	5/6	2.6(0.7-8.7)	0.87
level 2 drinker[a] and level 1 chewer	48/30	10.7(6.5-17.7)	0.58	54/30	6.3(2.9-10.2)	0.43
level 2 drinker and level 2 chewer	27/7	26.0(11.0-61.4)	0.39	33/7	14.8(6.3-33.8)	0.33
level 2 drinker and level 3 chewer	17/6	19.3(7.3-50.8)	0.86	17/6	9.2(3.5-24.1)	0.67
level 3 drinker[a] and level 1 chewer	17/9	11.4(4.9-26.5)	0.10	27/9	7.2(3.2-15.9)	0.47
level 3 drinker and level 2 chewer	**18/12**	**9.7(0.09-0.9)**	**0.04***	24/12	9.2(2.9-12.2)	0.29
level 3 drinker and level 3 chewer	**18/12**	**9.5(4.4-20.4)**	**0.008***	22/12	5.5(2.6-11.4)	0.08
level 4 drinker[a] and level 1 chewer	**10/7**	**8.6(3.0-24.3)**	**0.02***	**19/7**	**7.3(2.8-19.0)**	**0.04***
level 4 drinker and level 2 chewer	9/4	14.1(4.2-47.0)	0.09	19/4	12.1(3.9-36.5)	0.25
level 4 drinker and level 3 chewer	**18/8**	**14.6(6.1-35.0)**	**0.02***	**30/8**	**10.5(4.7-23.5)**	**0.04***

Abbreviations: CI: Confidence Interval; OR: Odds Ratio

Ca/Co: Case/Control; All OR's are adjusted for age (continuous), gender, region, socio-economic status and tobacco chewing.

[a]Cumulative alcohol consumption: level 1 drinker- ≤548.1 gram years; level 2 drinker- >548.1- ≤ 3380.31gram years; level 3 drinker- >3380.31- ≤9871.313 gram years; level 4 drinker- >9871.313 gram years.

[b]Cumulative chewing exposure: level 1 chewer- ≤ 100 years, level 2 chewer- 100-200 years, level 3 chewer- ≥200 years.

*Multiplicative interaction significant at α=0.05

Table 2.15. 5: Joint association risk estimates of heavy chewers at various alcohol drinking levels

Drinker levels	Chewer level	Oral cavity (N=950)			All HNC cases(N=1320)		
		Ca/Co	**OR (95%CI)**	**$P_{interaction}$**	**Ca/Co**	**OR (95%CI)**	**$P_{interaction}$**
Never drinker and Never chewer		154/1321	Reference		291/1321	Reference	
1	3	4/6	4.1(1.1-15.1)	0.39	5/6	2.6(0.7-8.7)	0.87
2	3	17/6	19.3(7.3-50.8)	0.86	17/6	9.2(3.5-24.1)	0.67
3	3	**18/12**	**9.5(4.4-20.4)**	**0.008***	22/12	5.5(2.6-11.4)	0.08
4	3	**18/8**	**14.6(6.1-35.0)**	**0.02***	**30/8**	**10.5(4.7-23.5)**	**0.04***

Abbreviations: CI: Confidence Interval; OR: Odds Ratio
Ca/Co: Case/Control; All OR's are adjusted for age (continuous), gender, region, socio-economic status and tobacco chewing.
[a]Cumulative alcohol consumption: level 1 drinker- ≤548.1 gram years; level 2 drinker- >548.1- ≤ 3380.31gram years; level 3 drinker- >3380.31- ≤9871.313 gram years; level 4 drinker- >9871.313 gram years.
[b]Cumulative chewing exposure: level 1 chewer- ≤ 100 years, level 2 chewer- 100-200 years, level 3 chewer- ≥200 years.
[*]Multiplicative interaction significant at α=0.05

Table 2.15. 6: Joint association risk estimates of heavy alcohol drinkers at various chewing levels

Chewer levels	Drinker level	Oral cavity (N=950)			All HNC cases(N=1320)		
		Ca/Co	**OR (95%CI)**	**$P_{interaction}$**	**Ca/Co**	**OR (95%CI)**	**$P_{interaction}$**
Never drinker and Never chewer		154/1321	Reference		291/1321	Reference	
1	4	**10/7**	**8.6(3.0-24.3)**	**0.02***	**19/7**	**7.3(2.8-19.0)**	**0.04***
2	4	9/4	14.1(4.2-47.0)	0.09	19/4	12.1(3.9-36.5)	0.25
3	4	**18/8**	**14.6(6.1-35.0)**	**0.02***	**30/8**	**10.5(4.7-23.5)**	**0.04***

Abbreviations: CI: Confidence Interval; OR: Odds Ratio
Ca/Co: Case/Control; All OR's are adjusted for age (continuous), gender, region, socio-economic status and tobacco chewing.
[a]Cumulative alcohol consumption: level 1 drinker- ≤548.1 gram years; level 2 drinker- >548.1- ≤ 3380.31gram years; level 3 drinker- >3380.31- ≤9871.313 gram years; level 4 drinker- >9871.313 gram years.
[b]Cumulative chewing exposure: level 1 chewer- ≤ 100 years, level 2 chewer- 100-200 years, level 3 chewer- ≥200 years.
[*]Multiplicative interaction significant at α=0.05

Table 2.16. 1: Association of socio-economic status with sub-sites of head and neck cancer adjusted for age, gender and region.

Parameters	Categories	HNC Primary sub-site												All HNC cases(N=1320)		
		Oral cavity (N=950)			Oropharynx(N=166)			Hypopharynx(N=177)			Larynx(N=86)					
		Ca/Co	OR (95%CI)	p value	Ca/Co	OR (95%CI)	p value	Ca/Co	OR (95%CI)	p value	Ca/Co	OR (95%CI)	p value	Ca/Co	OR (95%CI)	p value
Education	Nil, Illiterate	98/124	Reference		21/124	Reference		12/124	Reference		9/124	Reference		140/124	Reference	
	Literate	25/58	0.35(0.19-0.63)	<0.001	10/58	0.69(0.28-1.7)	0.43	5/58	0.56(0.17-1.7)	0.33	4/58	0.59(0.16-2.1)	0.42	44/58	0.44(0.27-0.73)	0.002
	< 5 years of schooling	73/93	0.60(0.38-0.93)	0.02	16/93	0.55(0.25-1.2)	0.13	15/93	0.77(0.32-1.8)	0.56	10/93	0.69(0.25-1.9)	0.48	114/93	0.64(0.43-0.96)	0.03
	5-8 years of schooling	248/369	0.50(0.35-0.70)	<0.001	60/369	0.62(0.34-1.1)	0.12	34/369	0.60(0.28-1.2)	0.18	28/369	0.65(0.28-1.5)	0.33	370/369	0.53(0.39-0.73)	<0.001
	High School	298/606	0.35(0.25-0.49)	<0.001	43/606	0.28(0.15-0.53)	<0.001	32/606	0.34(0.16-0.72)	0.005	24/606	0.33(0.14-0.80)	0.01	398/606	0.34(0.25-0.46)	<0.001
	College graduation or more	207/674	0.21(0.25-0.49)	<0.001	16/674	0.08(0.03-0.16)	<0.001	19/674	0.15(0.06-0.34)	<0.001	11/674	0.11(0.04-0.29)	<0.001	253/674	0.18(0.13-0.25)	<0.001
P_{trend}			<0.001			<0.001			<0.001			<0.001			<0.001	

Abbreviations: CI: Confidence Interval; OR: Odds Ratio

Ca/Co: Case/Control; All OR's are adjusted for age (continuous), gender and region.

Missing values are excluded from analysis.

Table 2.16. 2: Association of socio-economic status with sub-sites of head and neck cancer adjusted for age, gender, tobacco chewing, tobacco smoking pack years and alcohol gram years.

Parameters	Categories	HNC Primary sub-site												All HNC cases(N=1320)		
		Oral cavity (N=950)			Oropharynx(N=166)			Hypopharynx(N=177)			Larynx(N=86)					
		Ca/Co	OR (95%CI)	p value	Ca/Co	OR (95%CI)	p value	Ca/Co	OR (95%CI)	p value	Ca/Co	OR (95%CI)	p value	Ca/Co	OR (95%CI)	p value
Education	Nil, Illiterate	98/124	Reference		21/124	Reference		12/124	Reference		9/124	Reference		140/124	Reference	
	Literate	25/58	0.37(0.1-0.7)	0.003	10/58	0.63(0.2-1.6)	0.34	5/58	0.58(0.1-1.9)	0.37	4/58	0.6(0.1-2.2)	0.45	44/58	0.52(0.3-0.9)	0.02
	< 5 years of schooling	73/93	0.61(0.3-1.0)	0.052	16/93	0.53(0.2-1.1)	0.12	15/93	0.7(0.2-1.7)	0.44	10/93	0.71(0.2-1.9)	0.51	114/93	0.65(0.4-1.0)	0.05
	5-8 years of schooling	248/369	0.53(0.3-0.7)	0.002	60/369	0.58(0.3-1.0)	0.09	34/369	0.52(0.2-1.1)	0.10	28/369	0.65(0.2-1.5)	0.33	370/369	0.57(0.4-0.8)	0.002
	High School	298/606	0.45(0.3-0.6)	<0.001	43/606	0.32(0.1-0.6)	0.001	32/606	0.35(0.1-0.7)	0.008	24/606	0.36(0.1-0.8)	0.02	398/606	0.43(0.3-0.6)	<0.001
	College graduation or more	207/674	0.35(0.2-0.5)	<0.001	16/674	0.09(0.04-0.2)	<0.001	19/674	0.18(0.08-0.4)	<0.001	11/674	0.12(0.4-0.3)	<0.001	253/674	0.28(0.1-0.3)	<0.001
P_{trend}			0.84(0.78-0.89)	<0.001		0.67(0.60-0.75)	<0.001		0.72(0.63-0.83)	<0.001		0.69(0.59-0.80)	<0.001		0.78(0.74-0.83)	<0.001

Abbreviations: CI: Confidence Interval; OR: Odds Ratio

Ca/Co: Case/Control; All OR's are adjusted for age (continuous), gender, region, tobacco chewing, tobacco smoking pack years and alcohol gram years.

Missing values are excluded from analysis.

2.8 Discussion

In the present study, detailed analysis was performed to know the cause and estimate of various lifestyle exposures on risk of developing primary sub-sites of HNC- OC, OPX, HPX and LX. The strength of the study is that large number of study participants (1320 cases and 1924 controls) were enrolled in relatively short period of time with interviews conducted in a close room by trained investigators under constant supervision of senior staff. All the cases were histologically confirmed. The controls have been enrolled from a pool of visitors coming to TMH along with various cancer site patients. As the HNC sub-sites cases were enrolled from TMH, the selection of visitors as control, visiting TMH along with all cancer site patients in different units, group matched on age, area of residence and gender ensured that the selection bias is minimal. There was good correlation between main questionnaire and abbreviated questionnaire which was administered on approximately 4% of study participants indicating reliability of measured exposures. Only incident cases diagnosed not more than 6 months before the date of enrolment were enrolled to ensure that information on exposure given by the cases is not influenced because of long duration of illness and exposures related to survival. The constant monitoring of data at three levels helped to keep the missing information at minimum. The continuous training and preparation of manual ensured that information is collected similarly between cases and controls by different interviewers.

The case-control study design has however; inherited weakness as study participants are interviewed after the outcome has occurred and controls are not randomly selected from known population, leading to a possibility of recall and selection bias. In the present study, enrolment of controls from same study base, enrolling only incident cases, conducting interviews in closed room by trained investigators, constant monitoring, 4% re-sampling to measure reproducibility,

as discussed, have helped to keep these biases at minimum. A definite selection/inclusion criterion was used for cases and controls which was independent of the exposures to be studied to control for selection bias. Case control study design is also susceptible to information bias as the interviewers are not blinded to the case control status of the study participants. To minimize the bias a structured questionnaire was used with specific close ended questions to maximize accuracy and completeness. All the interviewers were trained to maintain same degree of questioning for cases and controls.

Confounding was controlled by matching cases and controls on potential confounders such as age, gender and region of residence at enrolment during design and implementation of the study. During data analysis stratification and adjustment with additional confounders such as alcohol drinking, tobacco chewing, tobacco smoking, socio-economic status was used to minimize confounding. However, residual confounding might still have remained after controlling for potential confounders, but we observed strong association between exposures and outcome in our study. Thus, residual confounding even if present might not affect the risk estimates significantly or change the direction of association.

The prevalence of tobacco use in males and females of study controls was 55.01% and 10.18% respectively. Alcohol use prevalence in male and female controls was 16.74% and 0.14% respectively. We compared these prevalences with National Family Health Survey (NHFS-4) data 2015-2016 [121]. Our study control population had similar tobacco use prevalence and slight lower alcohol prevalence than NHFS-4 data. We performed chi squared test to determine the difference in proportions is significant or not. Chi-squared (χ^2) p values for difference in proportions >0.05 for tobacco in both genders and for alcohol it was >0.05 in females but <0.05 in males. The possible reason for lower alcohol prevalence in our study than the national data

might be due to the social stigma attached to it especially in females. There is no significant difference in proportion of exposures inferring that there is minimum selection bias in our study and the study finding can be applied to the general population.

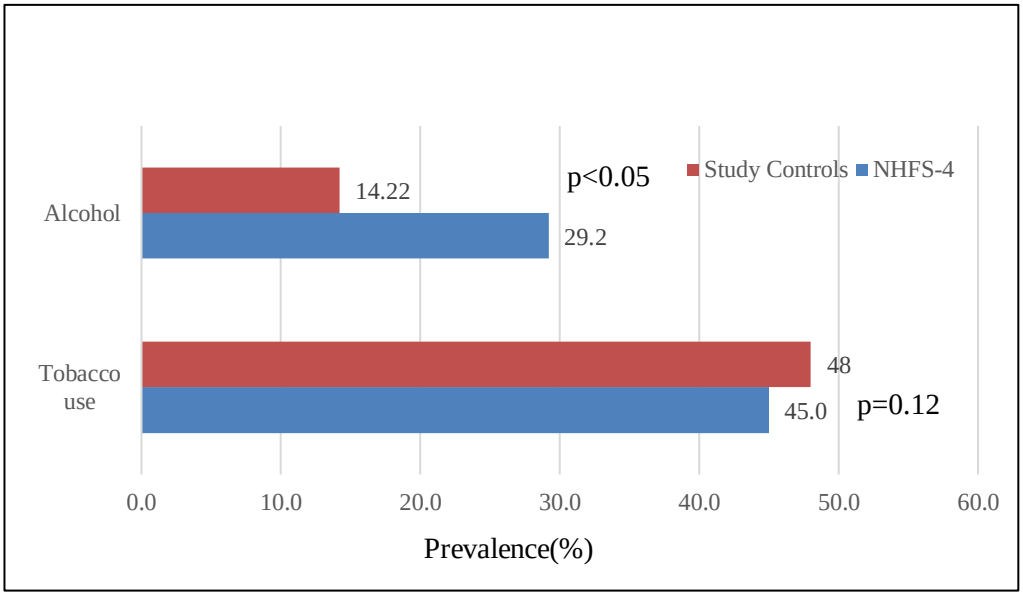

Figure 2. 2: Comparision of prevalence of exposures in males between study controls and NHFS-4 (age group 15-49).

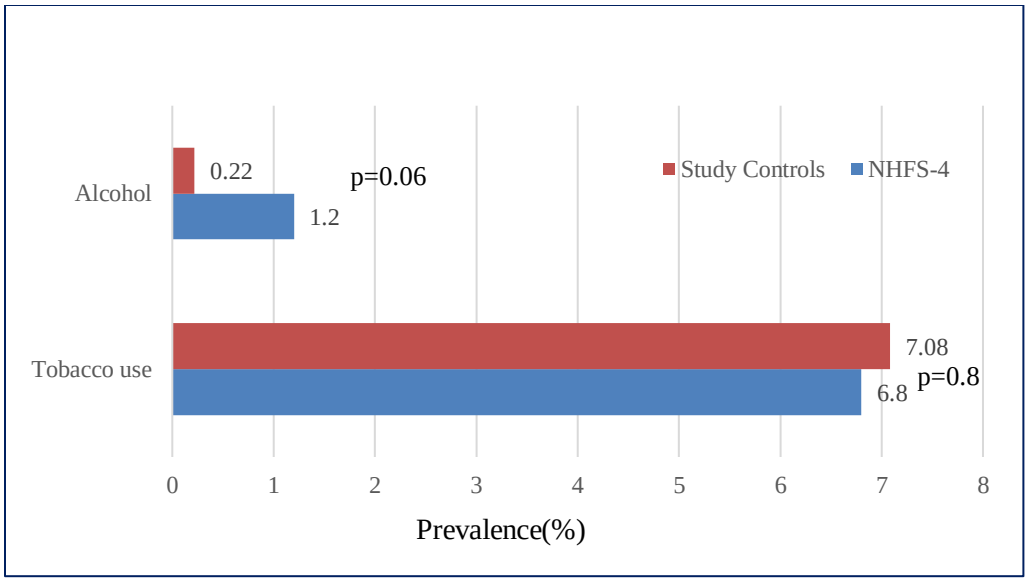

Figure 2. 3: Comparison of prevalence of exposures in females between study controls and NHFS-4(age group 15-49).

2.8.1 Tobacco smoking

Statistically significant association for 'ever' smokers was observed in all HNC primary sub-sites, the findings were similar with other case control and cohort studies[11],[5],[122]. Bidi smoking risk was found to be higher compared to cigarette smoking for all HNC primary sub-sites (p value for heterogeneity <0.001). Amongst HNC primary sub-sites taken into consideration for the present study, increased risk was observed for bidi smoking in oropharyngeal cancer (11.6 fold; p value for heterogeneity <0.001). Similar finding was observed by Dikshit RP and Kanhere S in a population based case-control study conducted in 2000 [11]. The Indian bidi contains only a small amount of tobacco dust rolled in a dried leaf of tendu *(Diospyrous malanoxylon)* or Temburni tree *(Diospyrous ebenum)*. In comparison to US cigarettes, the mainstream smoke of bidi contains a much higher concentration of several toxic agents such as hydrogen cyanide, carbon monoxide, ammonia, other volatile phenols, and

carcinogenic hydrocarbons such as benz(a)anthracene and benzopyrene. Bidi also delivers more nicotine than Indian cigarettes [96]. The nitrosonornicotine (NNN) and 4(methyl-nitrosoamino)-1-(3-pyridol) (NNK) level of bidi tobacco ranged from 6.2 to 12 µg/g compared with 1.3 to 58.0 µg/g in cigarette tobacco [11],[123]. Thus, the higher yields of tobacco specific nitrosamines (TSNA) in bidi smokers suggest the biological plausibility of higher risk observed in oropharyngeal cancer. The observed risk of smoking both bidi and cigarette as compared to never smokers was highest for Oropharynx cancer (OR=7.1; 95% CI 3.7-13.8), it was lower than expected suggesting less than multiplicative mode of action or those who smoke both maybe light smokers. Statistical significant interaction of cigarette and bidi smoking was observed for OPX, HPX and LX which holds true to the fact that these cancers are more attributable to smoking as compared to OC which is more attributable to chewing. This is the first ever case control study with large sample size in India to estimate statistical interaction of cigarette and bidi smoking for OPX, HPX and LX. Cumulative exposure of smoking was measured in pack years assuming that a cigarette contains 1gm and a bidi 0.5gm of tobacco. Analyzing the smoking risk by pack years has been used in many international studies [7]. A significant dose-response relationship was observed between increase in pack years and OPX, HPX and LX cancers. The increase in risk was almost 2-fold for OPX, HPX and LX cancers for every 500 pack years increase in smoking. A case control study conducted by Znaor et al [10] is the only study conducted in India till date which measures smoking risk of OC and pharyngeal cancers by pack years.

2.8.2 Tobacco chewing

'Ever' tobacco chewing was observed to have stronger risk for OC than rest of the HNC primary-sites. The study findings for risk of ever tobacco chewing were similar with other case-control studies conducted in India [10],[11],[9]. This is the first case control study to study the risk of different tobacco products in India. Amongst all tobacco products, *Gutka* chewing had highest risk for OC (p value for heterogeneity <0.001). It is hypothesized that *Gutka* has higher levels of heavy metals such as nickel, cadmium and arsenic and also which might increase its carcinogenic effects[124],[125],[126]. Alkaline pH level of *Gutka* is also hypothesized to increase its carcinogenicity. Chewing without tobacco i.e. areca nut and *Paan masala* has also found to have higher risk for OC, OPX and HPX (p value for heterogeneity >0.05). The risk estimates for chewing without tobacco were slightly higher compared to other case control studies conducted by Balaram et.al and Znaor et al. There are very few studies which study the risk of areca nut with respect to OC, OPX, HPX and LX. Ours is one of the few studies which studies the risk of areca nut and *Paan masala* (without tobacco) for oral cavity and pharyngeal cancers. In Indian context, areca nut chewing is less studied as an independent risk factor for head and neck cancers. Our study has observed areca nut chewing as a strong risk factor for HNC. Most of the studies couldn't find statistically significant association of areca nut chewing possibly due to limited sample size[18],[11],[127],[10]. In the Indian sub-continent mature areca nut is consumed. Nitrosamines classified as possibly carcinogenic to humans (Group 2B) by IARC are formed in saliva upon chewing areca nut[15]. *Khaini* and tobacco quid are forms of smokeless tobacco where slaked lime is added with tobacco. Consumption of slaked lime causes alkaline conditions which triggers formation of reactive oxygen species (ROS) which leads to oxidative damage of the DNA leading to carcinogenesis. Studies have shown that the calcium

hydroxide content in slaked lime was highly correlated to ROS production[128]. Addition of slaked lime also increases the pH level of the product which results in increased delivery and absorption of psychoactive ingredients such as arecoline [129]. Our study findings shed some light on carcinogenic effects of products without tobacco like areca nut, *paan* masala and lime. Therefore, simply advising to stop tobacco consumption in order to prevent cancer should not be incorporated in public health messages.

'Others with tobacco' category included tobacco containing dentifrices such as *gul, gudakhu and lal dant manjan.* A case control study conducted by Wasnik et. al in central India observed risk of 5.7 times for use of tobacco containing dentifrices and OPX cancer, the risk was unadjusted for possible confounders [18]. Our study however observed elevated risk of 8.5 for OPX cancer after adjusting for all possible confounders (age, gender, region of residence, socio-economic status, tobacco smoking and alcohol drinking) suggesting a strong association of tobacco containing dentifrices for pharyngeal cancers. Cumulative exposure to chewing was measured in chew years by multiplying number of times tobacco chewed per day and chewing duration, similar analysis was done performed by Znaor et al. [10]. Significant dose- response relationship was observed with increase in chew years and OC, OPX and HPX. Highest risk for OC was observed for chewing more than 11 times per day, whereas a lower risk was observed for chewing more than 20 times per day. This possibly could be explained by tooth loss which occurs after persistent years of chewing. Tooth loss thus might lower the frequency of chewing and may also result in change in oral micro-biota of the mouth. These factors might lower the risk for OC further.

A comparative analysis was performed for different types of tobacco products chewed and OC risk. This kind of analysis has never been reported in the past and is first of its kind. Amongst all

tobacco products chewed, *Gutka* chewing showed much higher risk for OC (p value for heterogeneity <0.05). A significant risk of 34-fold times more for chewing *Gutka* > 11 times/day is reported for first time. *Gutka* is a commercially manufactured tobacco product and contains additives such as heavy metals. There are few studies in India which study the toxic additives in the marketed SLT products. A study carried out by Dhaware et. al compared 13 brands of *Gutka* for its heavy metal content viz. lead (Pb), cadmium (Cb), Nickel (Ni), Copper (Cu) and Arsenic (As). Compared to other SLT products, *Gutka* had significant high levels of all heavy metals[130]. The study concluded that *Gutka* products have heavy metals above prescribed levels by WHO.

Areca nut and chewing products containing areca nut emerged as strong independent factor for HNC sub-sites especially for OC in our study. Areca nut extracts have been found to induce cytokine production by immobilizing Calcium in immune cells (T cells, mast cells and monocytes) which can contribute to chronic inflammation leading to carcinogenesis [131]. Arecoline is one of the major alkaloids found in areca nut. It has been found to stimulate collagen synthesis in fibroblasts[68]. Further research is necessary to improve our understanding of the basic biology, mechanisms, and epidemiology of areca nut use enabling us to design possible prevention and cessation programmes for areca nut users [129]. Our study findings shed some light on the deleterious effects of independent areca nut chewing and thus should be given equal importance in cancer prevention policies along with tobacco in India.

2.8.3 Alcohol drinking

Our results are in agreement with those of previous showing that alcohol consumption is an independent risk factor for development of HNC with a strong dose-response relationship[132],[77],[84],[79], [133]. Alcoholic beverages and acetaldehyde, the main

metabolite of ethanol, are classified as a class I carcinogen[134]. It is plausible that alcohol after being metabolized acts both directly and indirectly in HNC carcinogenesis by acting as a solvent for tobacco carcinogens [96], [135]. Ever alcohol drinking was observed to be significantly associated with OC and OPX cancers with OPX being most associated (p value for heterogeneity >0.05). Most of the studies have found OPX cancer to be strongly associated with alcohol consumption than OC cancer [21],[136],[137]. This suggests the biological plausibility that anatomical sites of pharynx are directly exposed to ingestion of alcohol similarly reported by other studies[135],[138].Ever alcohol drinking was not found to be associated with HPX and LX cancers. This may be due to inadequate number of cases of HPX and LX. Similar findings for HPX and LX were observed in Karungappally cohort study[85]. Amongst all alcohol types studied drinking beer and country spirit had higher risk for OC cancer (p value for heterogeneity >0.05). Drinking country spirit had stronger risk for OPX cancer (p value for heterogeneity >0.05). Risk estimates observed in our study for consumption of country spirit and OC, OPX cancer were similar to other case control studies (although few) conducted in India [10].Differential risk for HNC sub-sites was observed which is consistent with other studies. Our study cohort had higher proportion of whisky drinkers 50.4% whereas the proportion of country spirit drinkers was 42.8% amongst ever drinkers. Over the years in case control studies analysis by alcohol type has given inconsistent results and mostly the beverage consumed commonly in a study was observed to have greater risk in every study. This might be due to inadequate power to assess uncommon drinks, under reporting or misclassification of consumption[135].

A statistically significant dose-response relationship was observed between increase in duration of alcohol drinking years and risk of OPX cancer. Statistically significant risk estimates were observed at highest quartile of ethanol gram years for OPX and HPX cancers which were

comparatively higher than OC risk as observed in other studies. Analysis was also performed by categorizing the ethanol gram years of each alcohol type and estimating the risk for HNC sub-sites. The ethanol gram years of each alcohol type were categorized equivalent to drinking <120ml/day and >120ml/day for a whole year of that drink. As seen in Table 2.12. significant risk for drinking country spirit was observed for both categories (<120ml/day and >120ml/day for a year) for OC and OPX cancers and the risk was highest for OPX cancer in the higher category. This finding supports the biological plausibility as mentioned earlier.

Further studies can be designed to study alcohol etiology by using self-administered questionnaire which leads to minimum under-reporting.

2.8.4 Joint association between tobacco and alcohol drinking

Our study supports the evidence of more than multiplicative synergistic effect between alcohol consumption and tobacco smoking for OC and overall HNC as found in other studies [98],[21],[139],[140],[141][142]. The interaction effect is biologically plausible as alcohol can act as a solvent for carcinogens in tobacco smoke making the mucosa more susceptible, resulting in enhancement of carcinogenic properties of both exposures [135],[96]. We had low numbers of OPX, HPX and LX cases in strata to assess the interaction. Until now only 3 studies in India have assessed alcohol and smoking interaction, all conducted in South India[9], [10]. Our study however has representative participants from all regions, largely from North and Western India (Refer table 2.1).

We observed more than multiplicative effect for tobacco chewing and alcohol. Higher joint risk was observed for OC than HNC. The role of alcohol might be similar that its acts as solvent for SLT carcinogens which increases their absorption in the oral mucosa. There are very few studies who have investigated chewing and alcohol interaction in India. Study by Znaor et al have not

studied interaction with regards to cumulative exposure of both lifestyles. Thus, this will be a new finding from India.

2.8.5 Socio-economic status

Completed education of the study participant was used as proxy variable for socio-economic status in our study as it's more reliable measure of exposure than income level in India. the advantages to measure education as indicator for SES are that its relatively easy to measure, it is not loaded or controversial question as other SES measures such as level of income and is stable throughout life. Higher level of education was observed to have strong protective association for all HNC sub-sites after adjusting for necessary strong confounders like age (continuous), gender, region of residence, tobacco smoking pack years (continuous), tobacco chewing (categorical) and alcohol gram years (continuous). This finding is similar to previous studies conducted[26],[25],[87],[86]. The SES risk estimates may have been elevated since we could not rule out residual confounding of occupational exposure in our analysis as both exposures are highly correlated. However, in Indian context measuring occupational exposures is difficult due to heterogeneous nature of the occupations and it was beyond the scope of this analysis. The biological pathways between direct effects of SES and cancer development are not entirely clear, but emerging hypotheses include the effects of 'biological ageing' resulting from poor socioeconomic circumstances mediated by shortened telomeres[143],[144].

2.9 Summary

The strongest risk factors for HNC and its sub-sites after adjusting for potential confounders are as follows:

1. Bidi smoking is strongly associated with HNC sub-sites than cigarette smoking. Every 500 years increase in pack years doubles the risk of pharyngeal cancers and overall HNC compared to a never smoker.
2. Cigarette and bidi smoking have less than multiplicative synergistic effect in causation of HNC cancer. The synergistic effect is highest for OPX cancers.
3. Ever chewing has strongest risk for OC than pharyngeal cancers. Chewing without tobacco or areca nut chewing or also has strong association with HNC sub-sites particularly for OC.
4. *Gutka* chewing has very strong risk for OC amongst all tobacco products studied. Tobacco products with areca nut viz, betel leaf with tobacco, tobacco quid, *Gutka* and *Mawa* have higher risk for OC cancer which infers that areca nut acts synergistically with tobacco in causation of OC and overall HNC.
5. Areca nut chewing has independent role in association with OC and pharyngeal cancers. Along with tobacco, areca nut should also be given equal importance in prevention of HNCs.
6. Ever alcohol drinking is associated with OC and OPX cancers, more strongly for OPX cancer. Beer drinking is associated with OC and overall HNC. Whisky drinking is associated with OC and pharyngeal cancers.
7. Country spirit drinking is strongly associated for OPX cancer; it increases the risk by 2.5 times as compared to a never drinker.
8. Literacy and higher education are independently associated with HNC sub-sites.

CHAPTER 3
HUMAN PAPILLOMA VIRUS AND HEAD AND NECK CANCER

3.1 Introduction

In addition to lifestyle factors HNC risk can also be attributed to HPV. HPV is established risk factor for oropharyngeal cancer in developed countries. However, in India the prevalence and role of HPV in HNC development in still unclear. Papillomaviruses are small, non-enveloped, epitheliotropic, double-stranded DNA viruses that infect mucosal and cutaneous epithelia having genome of about 8000 base pairs (bp). Taxonomically they are classified into *genus* alpha, beta, gamma, delta, epsilon, zeta, eta and so on [145]. IARC has classified high risk (HR)- HPV types 16,18,31,33,35,45,52,58 and less commonly found 39,51,56,59 as Group 1 human carcinogens [146]. The test of choice for detecting HPV in clinical specimens are based on nucleic probe technology owing to well-known gene structure of the virus [146]. Many methods with different levels of analytical sensitivity and clinical specificity have been developed to detect the presence of high-risk (HR) types of (HPV) in clinical samples. Various techniques are in use for HPV DNA detection: (i) direct probe methods, such as Southern blotting and in situ hybridization, (ii) signal amplification methods, such as the hybrid capture 2 (HC2) assay, and (iii) target amplification performed by a variety of PCR-based techniques. For genotyping, PCRs are being followed by signal read-out methods, such as sequence analysis, restriction fragment length polymorphism analyses, or hybridization with type-specific probes by different formats, such as membrane-based reverse line blot (RLB) assay. Recently, several RLB assays based on different PCR protocols (MY09/11, SPF, or GP5+/6+). After PCR amplification, HPV sequences are detected by enzyme immunoassay

(EIA), and subsequent typing is performed by hybridization of the biotinylated PCR products to type-specific oligonucleotides immobilized on membranes followed by their

detection using an enhanced chemiluminescence reaction. Due to the format of the line blot strips, current assays are restricted to a maximum of about 40 oligonucleotide probes per hybridization reaction and depend upon visual read-out of the signal. Luminex (xMAP) suspension array technology is based on polystyrene beads with a diameter of 5.6microns that are internally dyed with various ratios of two spectrally distinct fluorophores. Thus, an array of 100 different bead sets with specific absorption spectra is created. Different molecules, such as individual oligonucleotide probes, can be coupled to different bead sets. These sets are combined to a suspension array and, due to their unique absorption spectra, allow up to 100 different probes to be measured simultaneously in a single reaction (multiplexing). In comparison to RLB, MPG appears to be more sensitive for the detection of HPV in GP5+/6+-PCR products from clinical samples, and it is suitable for epidemiologic and also diagnostic applications.[147]

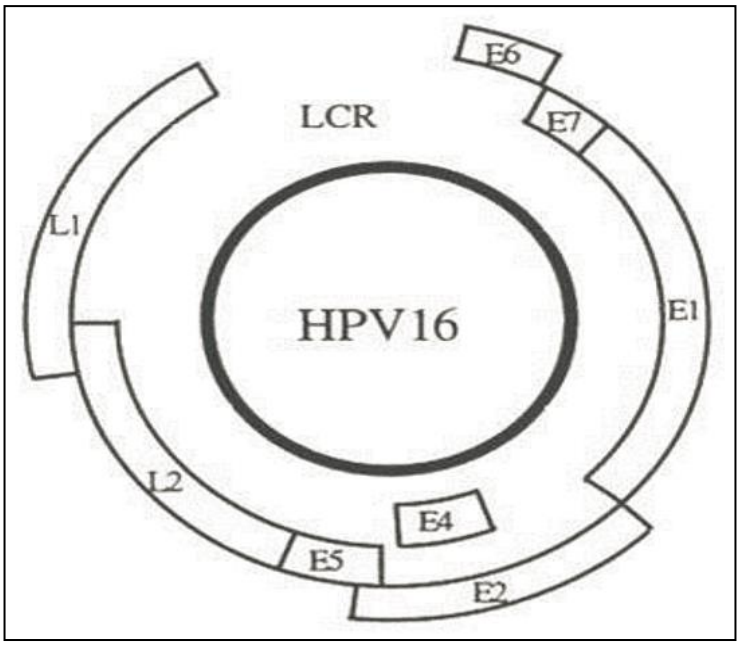

Figure 3. 1: Genome organization of HPV16

Location of the HPV major proteins: The HPV genome encodes early proteins with regulatory (E1 and E2) and transforming (E6 and E7) functions and two late capsid proteins (L1 and L2)

Table 3. 1: Benefits and weaknesses of the molecular methods for HPV detection

Method	Benefit	Weakness
Nucleic acid hybridization assay	• Southern blot is gold standard for HPV genome analysis • HPV detection is association with morphology	Low sensitivity, time consuming, cannot use degraded DNA
Signal amplification assays	• Quantitative • FDA approved test (hybrid capture 2) • Lower false positive rate • High sensitivity to genotyping	Licensed and patented technologies, wasn't designed to genotyping individual
Nucleic acid amplification assay	• Very high sensitivity • Multiplex analysis • Flexile technology (viral load and genotyping) • Detection of multiple infections	Lower amplification signals of some genotypes, Contamination may lead to false positives

3.2 Methodology

3.2.1 Tumour tissue collection and long archival storage

Tissue stabilization agent RNA later® was used to collect tissues. The tissues collected were either biopsy and surgically resected specimens. Approximately 0.5cm^3 size of tissue was collected in RNA later® and tubes were barcode labeled with study ID. The tissues were then stored at 4ºC overnight and processed the next day by pipetting out the RNA later® in sterile condition. The tissues were then immediately stored at -80ºC for long archival storage.

3.2.2 DNA extraction from tumour tissues

The tissues after removal from -80ºC freezers were subsequently thawed in 4ºC and then at room temperature to prevent tissue integrity and reduce heat shock. The tissues were cut into small

pieces with sterile blade and blade holder. Later the tissues were kept for overnight with intermittent shaking at 56ºC in tissue lysis buffer provided by Qiagen® DNAeasy Blood and Tissue kit. The later steps were followed as per kit manufacturer's instructions. Concentration of each DNA sample was determined by the optical density (OD) at 260 nm and the purification was evaluated by OD 260/280 ratio. Mean total yield of 166.93ug was obtained from 175 tumour tissue samples having 260/280 ratio range from 1.8-2.0. The yield was sufficient enough for genotyping.

3.2.3 Multiplex PCR for HPV genotyping

Qiagen Multiplex PCR kit was used for HPV PCR. The biotinylated primers were provided by the collaborator lab at IARC. The primers were specific for E7 region of the HPV genome and were developed based on conserved regions of the gene. The accession numbers of the GenBank sequences that we used references with the corresponding HPV types given in parentheses, were X05015 (HPV-18), X74479 (HPV-45), NC_001533 (HPV-51), M74117 (HPV-35), NC_001443 (HPV-58), M62849 (HPV-39), Y14591 (HPV-68), NC_001594 (HPV-56), NC_001695 (HPV-66), NC_001635 (HPV-59), M12732 (HPV-33), NC_001592 (HPV-52), K02718 (HPV-16), J04353 (HPV-31), X74472 (HPV-26), X74482 (HPV-53), U21941 (HPV-70), X94165 (HPV-73), and AB027021 (HPV-82) [39].

Table 3. 2: Sequences of forward and reverse HPV-type specific primers and sizes of the PCR-amplified fragments [34]

HPV type	Primer sequence[1]	PCR fragment size (base pair)
16	F.5'-TGAGCAATTAAATGACAGCTCAGAG-3' R. 5'-TGAGAACAGATGGGGCACACAAT-3'	212
18	F. 5'-GACCTTCTATGTCACGAGCAATTA-3' R. 5'-TGCACACCACGGACACACAAAG-3'	236
26	F. 5'-CGAAATTGACCTACGCTGCTACG-3' R. 5'-TGGCACACCAAGGACACGTCTTC-3'	239
31	F. 5'-AGCAATTACCCGACAGCTCAGAT-3' R. 5'-GTAGAACAGTTGGGGCACACGA-3'	210
33	F. 5'-ACTGACCTAYACTGCTATGAGCAA-3' R. 5'-TGTGCACAGSTAGGGCACACAAT-3'	229
35	F. 5'-CAACTGACCTATACTGTTATGAGC-3' R. 5'-TGTGAACAGCCGGGGCACACTA-3'	234
39	F. 5'-TTGTATGTCACGAGCAATTAGGAG-3' R. 5'-GACACTGTGTCGCCTGTTTGTTTA-3'	357
45	F. 5'-GACCTGTTGTGTTACGAGCAATTA-3' R. 5'-TGCACACCACGGACACACAAAG-3'	236
51	F. 5'-GCTACGAGCAATTTGACAGCTCAG-3' R. 5'-ATCGCCGTTGCTAGTTGTTCGCA-3'	242
52	F. 5'-ACTGACCTAYACTGCTATGAGCAA-3' R. 5'-CAGCCGGGGCACACAACTTGTAA-3'	229
53	F. 5'-ACCTGCAATGCCATGAGCAATTGAA-3' R. 5'-TTATCGCCTTGTTGCGCAGAGG-3'	253
56	F. 5'-ACCTACARTGCAATGAGCAATTGG-3' R. 5'-TGATGCGCAGAGTGGGCACGTTA-3'	244
58	F. 5'-GCTATGAGCAATTATGTGACAGCT-3' R. 5'-TGTGCACAGSTAGGGCACACAAT-3'	219
59	F. 5'-ACCTTGTGTGCTACGAGCAATTAC-3'	243

	R. 5’-GCTGCACACAAAGGACACACAAA-3’	
66	F. 5’-ACCTACARTGCAATGAGCAATTGG-3’ R. 5’-TGATGCGCAGAGTGGGCACGTTA-3’	244
68	F. 5’-TTGTATGTCACGAGCAATTAGGAG-3’ R. 5’-GATTACTGGGTTTCCGTTGCACAC-3’	258
70	F. 5’-CACGAGCAATTAGAAGATTCAGACA-3’ R. 5’-TTCCCGATGCACACCAGGGACA-3’	237
73	F. 5’-CTTACATGTTACGAGTCATTGGAC-3’ R. 5’-GTTTCTGGAACAGTTGGGGCAC-3’	221
82	F. 5’-GCTACGAGCAATTTGACAGCTCAG-3’ R. 5’-CATTGCCGATGTTAGTTGGTCGCA-3’	240

Abbreviation: [1]F: Forward; R: Reverse

Two primers for the amplification of β-globin (GenBank accession number AY260740) were added to provide a positive control for the quality of the template DNA [148]. To avoid cross-contamination quality control measures were taken viz. PCR master mix preparation Laminar Air flow hood (LAF) and final DNA addition LAF were kept physically separate, use of PCR strip tubes with caps were used and DNAse/RNAse free water was used as negative control for every PCR reaction.

3.2.4 Luminex assay for HPV genotyping

Luminex assay works in combination of following things: Optics, Fluidics and digital signal processing. 5.6microns polystyrene beads are used for multiplexing enabling analysis of 100 analytes per well. Bead serves as a solid phase for the molecular detection and each bead is assigned to an analyte. In HPV genotyping, each bead is coupled with specific probe for every HPV genotype. Coupled bead mix along with samples is kept for incubation at 95ºC to provide binding of the probe to the HPV DNA in the samples. The sample is sucked from the 96 well

filter plate by the syringe pump and transported to the cuvette. The beads are surrounded with sheath fluid which ensures a laminar flow between sample and the surrounding sheath fluid. So, each bead is singularized and precisely directed to focus laser detection system. Since spectral region is different from the others, it is possible to perform classification of beads and quantification of bound analyte at the same time. The advantages of this technology are that its high throughput, decrease in sample volume, reagents, labor and expense and easy to use tools for assay and data analysis. Detection limits range from 10 to 1,000 copies of the viral genome per reaction.

Following is the brief flowchart of Luminex assay methodology after performing HPV PCR

Add 10 ul of each biotinylated PCR product to each sample well of a AB900 plate. Add 10ul Tris-EDTA to blank well.

Vortex the tube containing beads. Add 40ul of beads to each sample well in dark condition. Cover the **reaction plate** with a plastic seal to prevent evaporation. Incubate in the oven at 95˚C for 15 minutes for DNA strand separation.

In the meantime, add 100ul wash buffer in each well of a **filter plate**. Wash buffer = 0.02% tween (100ul Tween 20 + 500ml Phosphate Buffer Saline). Incubate the filter plate for 30min at room temperature.

Place the **reaction plate** on ice for 2 min and then transfer subsequently to the pre-warmed Thermomixer. Incubate the **reaction plate** at 41˚C for 30 minutes under agitation 500rpm and protected from light.

Prepare dye in the meantime dilute Streptavidiin-R-phycoerythrin 1:1600. In a 15 ml flacon tube, Add 5500 ul of staining buffer (SB**=** 2M TMAC, 75mM Tris-HCl, pH = 8, 6mM EDTA, pH= 8.0, 1.5g/L sarkosyl**.) + 3.43ul** fluorochrome PE**.** Gentle and quick vortex, wrap the tube with foil and incubate in dark.

Aspirate the wash buffer from the **filter plate** on Milllipore filter plate vacuum wash station.

After 30minutes incubation of the reaction, take out the plate from thermomixer and transfer the samples from **reaction plate** to **filter plate** using multichannel. Wash the filter plate on Milllipore filter plate vacuum wash station.

Add 100ul of wash buffer. Remove the liquid by vacuum filtration. Shut the filter plate with the corresponding lid and remove all residual liquid from the bottom side by multiple vigorous blotting on a clean paper towel

Add 50ul of Streptavidin-Phycoerythrin dye to each sample well of the **filter plate**. Incubate protected from light at room temperature for 30 min under slight agitation (250rpm) on a horizontal shaker

Transfer the filter plate to the vacuum filtration manifold then remove the liquid by vacuum filtration. Add 100ul Wash Buffer to each well and remove the liquid by vacuum filtration. Repeat the wash step 3 times with 100ul Wash Buffer per well each.

Shut the **filter plate** with the corresponding lid and remove all residual liquid from the bottom side of the filer plate by multiple vigorous blotting on a clean paper towel. Pipette 100ul wash buffer to the filter plate.

Incubate for 2 min protected from light at room temperature under slight agitation at 250rpm on a horizontal shaker. Read the plate on Luminex 200 (Luminex Corporation, Austin, TX) analyzer after doing the necessary settings in the Bioplex manager software.

3.3 Quality assessment

Intra and inter assay validation measures were used for quality control of the Luminex assay. Every Luminex 96-well plate was designed to run one sample in duplicate (intra- assay

validation) and one sample was run from previous assay (inter- assay validation). Tris-EDTA buffer was used as blank and PCR negative control was run as Luminex negative control.

3.4 Cut off calculation to determine HPV infection status

For each probe, the median fluorescence intensity (MFI) values obtained when no PCR product was added to the hybridization mixture in Luminex assay were considered the background values. The cutoff was computed by adding 5 MFI to 1.1 X the median background value of that specific HPV probe as done by the Schmitt et. al who developed the assay [149]. After every plate read the cut off were calculated to determine the HPV genotype presence.

3.5 Statistical analysis

A sample was considered as HPV positive if the MFI levels were above the cut off levels of that specific HPV genotype. The proportion of cancers caused by HPV and 95% confidence interval for the estimate are reported. Exact Chi-squared (χ^2) test was used to determine odds ratio of HPV positivity in presence of different lifestyle exposure such as smoking, chewing and alcohol drinking. The analysis was performed on STATA 15.0 (StataCorp, College Station, TX) statistical package and all reported P values are two sided. Statistical significance was set at $p < 0.05$ [38].

3.6 Results

HPV positive study participants belonged to younger age groups as compared to HPV negative study participants. They had lower proportion of tobacco chewers, higher proportion

of tobacco smokers and alcohol drinkers and late stage carcinomas than HPV negative participants. The overall HPV prevalence in HNC was 39.43%; the prevalence sub-site wise was OC- 36.27%; OPX-50%; HPX-50% and LX-26.32%. The most prevalent HPV genotype was HPV16 amongst all HNC sub-sites. HPV16 prevalence was highest in OPX and HPX (38.89%). HPV51 was most prevalent in HPX (11.11%) and HPV52 is most prevalent in LX (5.26%). However, HPV51 and HPV52 were borderline positives in all assays, on repeating they were borderline negatives. Thus, their prevalence should be treated with caution. HPV genotype co-infections were also observed. HPV co-infections of HPV16-HPV51, HPV16-HPV58 and HPV16-HPV58-HPV82 were observed. HPV16-58 co-infection was observed in all HNC sub-sites, most prevalent in OPX (11.11%). HPV16-HPV51 co-infection was observed in OC and OPX, most prevalent in OPX (2.78%). HPV16-HPV58-HPV82 co-infection was observed in LX only.

On performing Chi-square test for differences in HPV proportions across HNC sub-sites, there was no statistically significant difference found within the HNC subs-sites (Table 3.7)

Lifestyle exposures smoking (ever/never), chewing (ever/never) and alcohol drinking (ever/never) were not found to be statistically significant with HPV positivity.

We extracted p16 protein information from hospital medical records. Out of all OPX cases positive for HPV 2 cases were positive for p16, 14 cases were negative for p16 and information for the rest of the cases was not available.

Table 3. 3: Characteristics of study participants for Luminex HPV genotyping

Parameters	Categories	*Human Papilloma virus* (HPV); N=175			
		Positive(N=69)		Negative(N=106)	
		Number	%	Number	%
Age at Enrolment	18-29	5	7.2	6	5.66
	30-39	7	10.14	20	18.87
	40-49	16	23.19	17	16.04
	50-59	23	33.33	28	26.42
	60-69	14	20.29	25	23.58
	70-79	3	4.35	9	8.41
	80-89	1	1.45	0	0
	Missing	0	0	1	0.94
	Mean (±SD)	46.02(±15.8)		46.38(±16.11)	
Region of residence at enrolment	North	28	40.5	28	26.42
	West	25	36.2	50	47.17
	South	1	1.45	1	0.94
	East	9	13.04	17	16.04
	Central	4	5.8	10	9.43
	Missing	2	2.9	0	0
Gender	Males	61	88.41	85	79.44
	Females	8	11.59	20	18.6
	Missing	0	0	2	1.87
Any tobacco use	Never	2	2.9	11	10.38
	Ever	60	86.96	90	84.91
	Missing	7	10.14	5	4.72
Tobacco chewing	Never	20	28.9	25	23.58
	Ever	43	62.32	76	71.7
	Missing	6	8.7	5	4.72
Tobacco smoking	Never	33	47.83	68	64.15
	Ever	29	42.03	33	31.13
	Missing	7	10.14	5	4.72
Alcohol drinking	Never	43	62.32	73	68.87
	Ever	20	28.99	28	26.42
	Missing	6	8.7	5	4.72
Stage	1	1	1.45	5	4.7
	2	4	5.8	22	20.75
	3	16	23.19	23	21.7
	4	37	53.62	44	41.51
	Missing	11	15.94	12	11.32

Table 3. 4: Distribution of *Human Papilloma virus* genotypes in Oral cavity and Oropharynx sub-sites of head and neck cancer.

Parameter	Categories	Oral cavity(N=102)			Oropharynx (N=36)		
		Number	%	95% CI	Number	%	95% CI
Any HPV		37	36.27	0.27-0.46	18	50	0.32-0.67
HPV genotype independent	HPV16	29	28.43	0.19-0.38	14	38.89	0.23-0.56
	HPV51[a]	8	7.8	0.03-0.14	3	8.33	0.01-0.22
	HPV52[a]	1	0.98	0.0-0.05	0	0	NA
HPV genotype co-infection	HPV16-58	9	8.82	0.04-0.16	4	11.11	0.03-0.26
	HPV16-58-82	0	0	NA	0	0	NA
	HPV16-51[a]	1	0.98	0.0-0.05	1	2.78	0.001-0.14
	HPV51-52[a]	1	0.98	0.0-0.05	0	0	NA

Table 3. 5: Distribution of *Human Papilloma virus* genotypes in Hypopharynx, Larynx and overall Head and neck cancer.

Parameter	Categories	Hypopharynx (N=18)			Larynx (N=19)			All HNC cases (N=175)		
		Number	%	95% CI	Number	%	95% CI	Number	%	95% CI
Any HPV		9	50	0.26-0.74	5	26.32	0.09-0.51	69	39.43	0.32-0.47
HPV genotype independent	HPV16	7	38.89	0.17-0.64	3	15.79	0.03-0.39	53	30.29	0.23-0.37
	HPV51[a]	2	11.11	0.01-0.34	1	5.26	0.001-0.26	14	8.0	0.04-0.13
	HPV52[a]	0	0	NA	1	5.26	0.001-0.26	2	3.77	0.001-0.04
HPV genotype co-infection	HPV16-58	1	5.56	0.001-0.27	1	5.26	0.001-0.26	15	8.57	0.04-0.13
	HPV16-58-82	0	0	NA	1	5.26	0.001-0.26	1	0.57	0.0-0.03
	HPV16-51[a]	0	0	NA	0	0	NA	2	1.14	0.001-0.04

Abbreviations: CI- Confidence interval; NA- Not applicable

[a]HPV51 and HPV52 were borderline positives. Hence their prevalence should be treated with caution.

Table 3. 6: Lifestyle factors and *Human Papilloma virus* prevalence.

Parameter	Categories	*Human Papilloma virus*		OR (95%CI)	p value
		Negative; N=106 (%)	Positive; N=69 (%)		
Smoking	Never	68 (64.1)	33(47.8)	Reference	
	Ever	33(31.1)	29(42)	1.81(0.90-3.6)	0.09
Chewing	Never	25(23.5)	20(28.9)	Reference	
	Ever	76(71.6)	43(62.3)	0.7(0.34-1.4)	0.3
Alcohol drinking	Never	73(68.8)	43(62.3)	Reference	
	Ever	28(26.4)	20(28.9)	1.2(0.58-2.5)	0.6

Abbreviations: CI: Confidence interval; OR: Odds Ratio.
The OR's are adjusted for age (continuous), gender and region.

Table 3. 7: Differences in HPV proportions across HNC sub-sites

HNC sub-sites (N)	HPV positive proportion	p value
Oral cavity (102)	36.27%	0.60
Oropharynx (36)	50%	0.24
Hypopharynx (18)	50%	0.38
Larynx (19)	26.32%	0.26
All HNC cases	39.43%	--

Table 3. 8: Details of HPV positivity in non-tobacco users

HPV genotype	HNC sub-site	TNM Stage	Tumour histology
HPV16	Oropharynx	T4N1M0	Moderately differentiated carcinoma, p16 negative
HPV51-52[a]	Oral Cavity	T1N2M0	Moderately differentiated carcinoma with metastatic regional lymph nodes

[a]HPV51 and HPV52 were borderline positives. Hence their prevalence should be treated with caution.

3.7 Discussion

In this prevalence study, we examined presence of 23 genotypes of HPV in head and neck cancers from fresh frozen tumour tissue which is largest till date in India. Tumour tissues are relatively better biological specimens than serum or plasma to analyze current HPV infection. The proportion of HPV attributable to HNC worldwide is significantly heterogeneous across by cancer anatomic site, geographic location and calendar time[150]–[153].Although HNC incidence is very high in India, only a limited number of studies have investigated the prevalence of HPV infection in HNC's [154]–[159].In these studies, the HPV viral DNA was determined by using PCR based assays and the HPV positivity varied from 15% to 70%. Many independent studies have highlighted the fact that PCR based assays are insufficient and inaccurate to establish viral causality[160], [161]. Owing to their high sensitivity these assays detect viral DNA traces which may not be linked to the carcinogenesis. A recent Indian study by Bhosale et. al detected a very low prevalence of HPV16 DNA (1.6%) using nested PCR method [162]. The substantial differences in the HPV16 DNA positivity in comparison with our study could be explained by the sensitivity of detection methods used. In our study we used a type specific multiplex HPV genotyping (TS-MPG) assay, which combines multiplex PCR and bead-based Luminex Technology (Luminex Corporation, Austin, TX). This assay was developed by IARC. A recent study by Gheit et al conducted in central India determined HPV DNA, RNA and positivity in retrospectively collected tumour tissue specimens. They found HPV DNA positivity of 13.7% in overall HNC, HPV16 positivity was 72% in all DNA positive tumours. However the study concluded that $p16^{INK4a}$ is not a good surrogate marker of HPV transformation in Indian HNC cases which is contrasting to European studies [92]. The study results question

the fact that whether universal algorithm of $p16^{INK4a}$ staining with HPV DNA detection should be adopted for Indian scenario.

In our study we found HPV prevalence of 39.43% in overall HNC in tumour tissue which was similar to case control study conducted by Anantharaman et al on subset of 120 tumours. They also performed HPV antibodies detection from plasma and got an agreement of 67% between HPV16 E6 serology and HPV E7 DNA based detection[29]. In this study, of all HPV HNC positives we found 76.8% independently positive for HPV16 DNA. Presence of HPV16 DNA, although necessary does not establish causality as it might be due transient infections. A study by Torre et. al reported similar HPV DNA and HPV16 DNA prevalence in overall HNC using Hybrid Capture 2 technology[163]. We also found samples borderline positives for HPV51 and HPV52 which are reported for the first time in India. We need to further confirm the presence of these genotypes by targeted sequencing. Our study also found multiple co-infections of HPV16-51, HPV16-58, HPV16-58-82 and HPV51-52 which have been also never reported in Indian population. However, these findings need to be validated on larger sample size. The sample which was positive for HPV51-52 co-infection was never user of tobacco and alcohol. This might be interesting finding and can be explored further. In our study HPV positive individuals had higher proportion of smokers which suggests the role of smoking related etiology in the HNC carcinogenesis as observed in previous studies. We found no significant risk of smoking, chewing and alcohol drinking with HPV positivity in our study.

Statistically there was no significant difference found of HPV proportions within HNC sub-sites. This may be due to limited sample size or might suggest the fact that HPV infection is

opportunistic and doesn't favor any HNC sub-site. A larger sample size will be able to answer this question.

3.8 Summary

To summarize our study found relatively high prevalence of HPV in HNC. This study is 2nd of its kind and the only study from western region of India to screen the presence of 23 HPV genotypes (high risk and low risk). Owing to the heavy burden of tobacco chewing and smoking in Indian HNC's the role of HPV in association with these cancers and its interaction with the said lifestyle exposures remains unknown in Indian population. Our study provides insights into the contribution of mucosal HPV genotypes types in the development of HNC and highlights important differences between published data from developed parts of the world and our own data from a major referral centre in India. However, we did not find any statistical differences in HPV proportions within HNC sub-sites. Also, at the same time it will be difficult to conclude that there aren't any statistical differences until we perform in on a larger sample size.

CHAPTER 4
CONCLUSION AND FUTURE PERSPECTIVES

4.1 Conclusion

The HNC incidence is increasing in India and recent estimates suggest that its common malignancy in Indian males after lung cancer. Lifestyle exposures such as tobacco chewing, tobacco smoking, alcohol drinking and education are found to be associated with HNC and its sub-sites. Smokeless tobacco use has wide heterogeneity in India owing to the regional differences. Tata Memorial Hospital being a primary referral centre in the country, there is large influx of patients coming from all regions of India. Hence this case-control was undertaken to identify the risk of all these lifestyle exposures stratified by the type and duration of each exposure. The major highlights of the case-control study were as follows (Table 4.1):

The HNC case control study enrolled 1320 HNC cases consisting of sub-sites (OC, OPX, HPX and LX) and 1924 visitor controls during same time period. The case to control ratio was ~1.4. The questionnaire data was obtained on major lifestyle exposures tobacco smoking, tobacco chewing, alcohol drinking and socio-economic status. The major lifestyle factors associated with HNC subs-sites after adjusting for potential confounders are as follows:

1. Ever smoking has higher risk for pharyngeal cancers (OPX, HPX and LX) than OC. The risk if strongest for OPX cancer and its almost double as that for LX cancer.
2. Bidi smoking has higher risk for pharyngeal cancers than OC and the risk is strongest for OPX cancer.
3. Cumulative exposure of smoking was measured in smoking pack years. A clear dose-response relationship was observed with increase in pack years of smoking for all pharyngeal cancers.

4. Cigarette and bidi use have statistically significant less than multiplicative synergistic effect on HNC subs-sites. The risk is highest for OPX cancer.
5. Ever tobacco chewing has highest risk for OC cancer and the risk is almost 4 times more than OPX and HPX cancer.
6. Chewing tobacco products like *Betel quid* with tobacco, tobacco quid, *Gutka, Khaini, Mawa , Mishri, gul, gudhakhu, laal dantmajan* has highest risk for OC cancer.
7. For OC and OPX cancer, out of all the tobacco products chewed *Gutka* has very strong risk. *Khaini* chewing has stronger risk for HPX cancer amongst all other smokeless tobacco.
8. Chewing products without tobacco like areca nut and *Paan masala* has stronger risk for OC cancer than OPX and HPX.
9. The risk starts before 5 years of chewing for OC cancer, whereas it starts after 10 years chewing for OPX and HPX cancer.
10. Chewing tobacco more than 10 times a day has highest risk for OC and overall HNC cancer.
11. The risk for chewing *Betel quid* with tobacco, tobacco quid, *Gutka, Khaini, Mawa, gul, gudhakhu, laal dantmajan* starts at less than 5 years of chewing for OC cancer.
12. *Mishri* and areca nut chewing duration risk starts after 5 years of chewing for OC cancer.
13. The risk for OC cancer starts at less than 10 times/day chewing of *Betel quid* with tobacco, tobacco quid, *Gutka, Khaini, Mawa, Mishri, gul, gudhakhu, laal dantmajan* and also non-tobacco products like areca nut.

14. Tobacco products with areca nut have higher risk for OC and pharyngeal cancers than products without areca nut indicating that areca nut acts synergistically with tobacco in causation of HNC cancer.
15. Our study found ever alcohol drinking to be a risk factor for OC and OPX cancer. Drinking beer, whisky and country spirit is associated with OC and OPX cancer. However, the sample size was insufficient to determine the association for HPX and LX cancer.
16. Country spirit drinking has highest risk for OC and OPX amongst all other types of alcohol. Statistically significant risk was observed for highest quartile of alcohol gram years of drinking.
17. Literacy confers protection against cancers of OC after adjusting for confounders like tobacco and alcohol. High school education and above shows protective association for pharyngeal cancers.

Along with tobacco and alcohol lifestyle exposures HPV has also being gaining importance in causation of HNC's worldwide especially for OPX cancers. India having heavy burden of HNC has few studies to estimate the role of HPV in Indian HNC cases. Hence, we also performed HPV detection and genotyping to study the prevalence of HPV genotypes in tumour tissue specimens of HNC cases. Following were the highlights of the study:

1. Our study found high prevalence of HPV in overall HNC with HPV16 being the most dominant genotype. OPX and HPX cancer had highest HPV prevalence with HPV16 being most dominant in them.
2. We also found borderline positives for HPV51 and HPV52 however their positivity needs to be validated.

Table 4. 1: Risk factor summary for HNC and its sub-sites derived from current study

Risk factor	Direction of effect
Association probably causal	
Tobacco smoking for OC, OPX, HPX,LX and overall HNC	↑↑
Cigarette smoking for OPX and HPX	↑↑
Bidi smoking for OC, OPX, HPX, LX and overall HNC	↑↑
Increased pack years of smoking for OC, OPX, HPX, LX and overall HNC	↑↑
Tobacco chewing for OC, OPX, HPX and overall HNC	↑↑
Increase in chewing duration for OC	↑↑
More than 10 times/day tobacco chewed for OC	↑↑
Increased chew years for OC, OPX, HPX and HNC	↑↑
Increase in chewing duration and times of chewing/day for BQ+T, *Gutka, Khaini, Mawa, Mishri, areca nut without tobacco gul and gudhakhu* for OC cancer	↑↑
Country spirit drinking for OPX cancer	↑↑
Ethanol gram years of >9871.313 for OPX cancer	↑↑
Literacy for OC cancer	↓↓
High school and above education for OPX, HPX and LX cancer	↓↓
Weak association probably causal	
Increase in chewing duration for OPX and HPX	↑
More than 10 times/day tobacco chewed for OPX and HPX	↑
Ever alcohol drinking for OC, OPX and HNC	↑
Beer, whisky and country spirit drinking for OC	↑
Whisky drinking for OPX	↑
Increase in ethanol gram years of drinking for OC	↑
Association uncertain	
Joint association of cigarette and bidi smoking for OC cancer	--
Ever tobacco chewing and LX cancer	--
Mawa and *Mishri* chewing for OPX cancer	--
Mawa chewing for HPX cancer	--
Gutka, Khaini, Mawa and Mishri, areca nut without tobacco chewing and LX cancer	--
Ever alcohol drinking and LX cancer	--

Abbreviations: ↑↑ high to moderate increase in the risk; ↑ slight increase in the risk

↓↓ High to moderate decrease in the risk; -- Association uncertain

4.2 Future perspectives

This study demonstrates that HNC and its sub-sites are preventable in India as the lifestyle exposures causing them are modifiable. Public health authorities should spread a strong message about harmful effects to tobacco and alcohol habits. The study demonstrated risk of different smokeless tobacco products and HNC and areca nut chewing also emerged as a strong risk factor for OC cancer. This study can form a base as studying regional variations in smokeless tobacco use across India which will help to design targeted prevention policies for HNC. Tobacco products which are mainly used for application such as *Mishri, gul, gudakhu and laal dantmanjan* also have been found to be strongly associated with OC cancer. The tobacco control program in India should be modified instead of giving out generic message to the masses. The smokeless tobacco products in India vary largely in chemical and heavy metal content. It has been hypothesized that these heavy metals in tobacco products also act synergistically in the tobacco carcinogenesis leading to HNC. This study observed variation in risk of different SLT products commercially manufactured in India. Owing to the complexity and variety of chemical ingredients contained in SLT products marketed in India, there should a uniform national database of chemical compositions and carcinogens of each form of SLT. This will help to strengthen the anti-tobacco campaigns and help better understanding of the tobacco epidemic in India.

Alcohol drinking has also been found as an independent risk factor for HNC especially for OPX. Future studies in should include other types of alcohol in larger sample sizes which are regionally brewed in India to have a comprehensive understanding of alcohol aetiology.

Future studies can be planned to take into consideration other indicators of socio-economic status like number of people living in a family, water supply source, and expenditure on different food items along with education to generate a matrix or scoring system to have better understanding of HNC aetiology.

High HPV prevalence was found in our study. This provides novel insights into HPV contribution to HNC's in India. Future studies should be planned on larger sample sizes with techniques to validate HPV prevalence like p16 Immunohistochemistry (IHC), HPV E6/E7 mRNA expression. Genome wide association studies should be planned to investigate the role of genetic susceptibility in Indian HNC scenario.

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