CHAPTER-1

INTRODUCTION

1.1 The Head and Neck Cancer: Overview

Head and neck cancer (HNC) is a broad term that refers to a heterogeneous group of malignancies that arise in the oral cavity, larynx, pharynx, nasal cavity and paranasal sinuses**.** Squamous cell carcinoma (SSC) is the most common histological type of head and neck malignancy, accounting for more than 90% of all head and neck cancers. Cancer of oral cavity and oropharynx are the two most common head and neck cancers. All the cancers in these head and neck areas have been more or less same epidemiology, aetiology and clinical presentation. (Argiris et al. 2008, Parkin et al. 2005). Higher incidence rate of head and neck squamous cell carcinoma (HNSCC) was found in males compared to females. The tobacco smoking, tobacco and betel quid chewing and alcohol drinking have been considered as major risk factors for HNSCC. On the other hand, recent epidemiologic studies indicate that the human papillomavirus (HPV) is a causative agent for some HNSCC and an independent risk factor for oropharyngeal squamous cell carcinoma (OPSCC). In addition, both genetic and epigenetic alternation also plays a crucial role towards the development of HNSCC (Pezzuto et al. 2015, Ragin et al. 2007).

Head and neck cancers are categorized based on the region of the head or neck in where cancer arise (**Figure 1.1**). The different areas of head and neck cancers are described below:

I. **Oral cavity**: The oral cavity covers the areas of the lips, tongue, gums, the lining inside the cheeks, the floor (bottom) of the mouth under the tongue, the hard palate (bony top of the mouth), and the part of the gum behind the wisdom teeth. The other most common cancer regions of oral cavity are lip, mouth floor, tongue, mucosa of buccal cavity, hard palate, mandibular gingiva and maxillary gingival.
II. **Pharynx**: The pharynx is a hollow tube about 5 inches long that begin behind the nose and guide to the esophagus. It includes three parts: the upper region of the pharynx behind the nose (**nasopharynx)**; the middle part of the pharynx, having the soft palate, the base of the tongue, and the tonsils **(oropharynx**); and the lower part of the pharynx (**hypopharynx**).
III. **Larynx**: The larynx (voice-box) is a short tube formed by cartilage just beneath the pharynx in the neck. The larynx has the vocal cords. It also

contains a small piece of tissue name as the epiglottis, which moves to wrap the larynx to impede food from getting into the air passages.

IV. **Paranasal sinuses and nasal cavity**: The paranasal sinuses are small void spaces in the area of the head surrounding the nose, whereas; the nasal cavity is the hollow space within the nose.

V. **Salivary glands**: The principal salivary glands are found in the floor of the mouth and close to the jawbone.

However, cancers of the thyroid gland, brain, eye, oesophagus, and those of the scalp, skin, bones, and muscles of the head and neck area, are not usually considered as head and neck cancers.

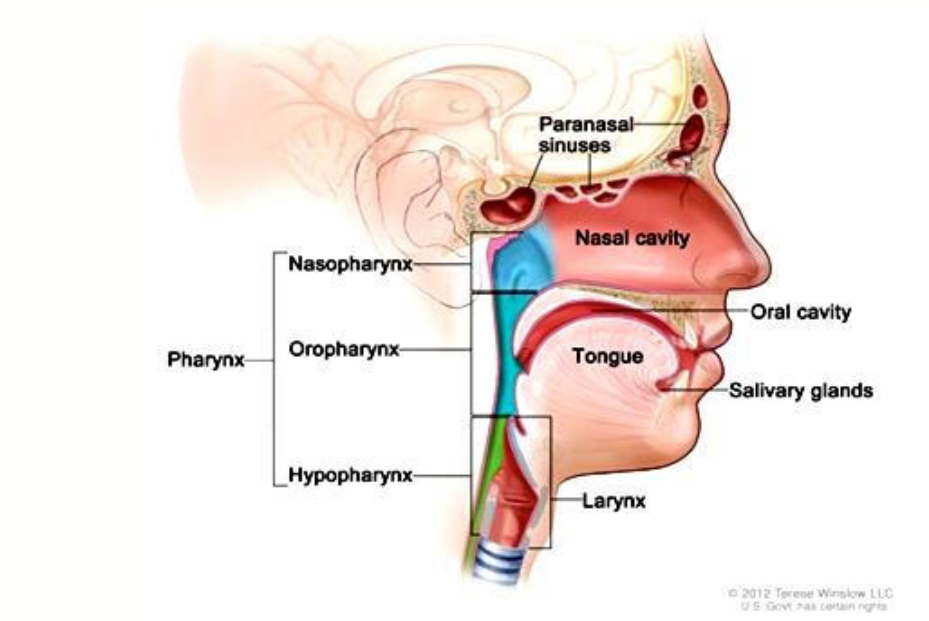

Figure 1.1: Diagrammatic representations of head and neck areas.

1.2 Epidemiology of Head and Neck Cancer

1.2.1 Global Incidence and Mortality

Globally, HNSCC is the sixth most frequent malignancy, accounting for more than 650,000 new cases each and 350,000 deaths every year (Parkin et al. 2005). International trends of HNSCC incidence rates showed significant heterogeneity, for example in India; oral cavity cancer is the most common cancer in males, however the rates of oral cavity cancer decreased for males and females in

Canada and the United States. Incidence rates of oropharyngeal cancer increased among both men and women in many European countries as well as some in Asian countries (Japan and India [Chennai]). However, oropharyngeal cancer rates declined among men and women of Hong Kong (China) and Mumbai (India). Incidence rates of larynx declined for men and women in nine countries and trends changed by sex in other areas. These variations in incidence rates across countries by sex, probably reflect diverge prevalence of well-known risk factors for HNC. For many tobacco-related HNC, incidence rates increased in countries where tobacco use remains common (e.g., in some European and Asian countries). The five-year overall survival rate of HNC patients is about 40-50% with a poor post treatment results. Over the past 30 years, survival rates for HNC have remained comparatively stagnant (Bose et al. 2013, Simard et al. 2014).

The worldwide incidence and mortality rate estimated from 27 different cancers and data of all these cancers is available in the (GLOBOCAN) database series of the International Agency for Research on Cancer (IARC). According to GLOBOCAN database, head and neck cancers accounting for 686,328 cases in 2012 (2.1% of the world total), which comprising 300,373 lip and oral cavity cancers, 156,877 larynx, 142,387 pharynx and 86,691 nasopharynx cancers with two-thirds occurring in men (Pezzuto et al. 2015). The incidence rates were quite high in men in the region of South-Central Asia and in Central and Eastern Europe. However, the region with the highest incidence among both males (22.9 per 100,000) and females (16.0 per 100,000) was Melanesia (a subregion of Oceania, northeast of Australia). Worldwide, 145,000 deaths occurred due to oral cancer (1.8% of the world total), of which 77% were in the less developed regions. Larynx cancer accounted for 157,000 new cases (1.1% of new cancers), is a cancer that is remarkably frequent among men, where it comprises 1.9% of male cancer cases. The male and female ratio is 7:1 and which is greater than any other site. It is a rare cancer in women with merely 19,000 new cases estimated in 2012. In men, the regions of high-risk for laryngeal cancer were the Caribbean and Central and Eastern Europe (7.9 per 100,000), Southern Europe (7.2 per 100,000) and Western Asia (6.5 per 100,000). Laryngeal cancer is responsible for 83,000 deaths in 2012 (1.0% of cancer deaths), of which 73,000 occurred in men (1.6% of deaths) (Ferlay et al. 2015).

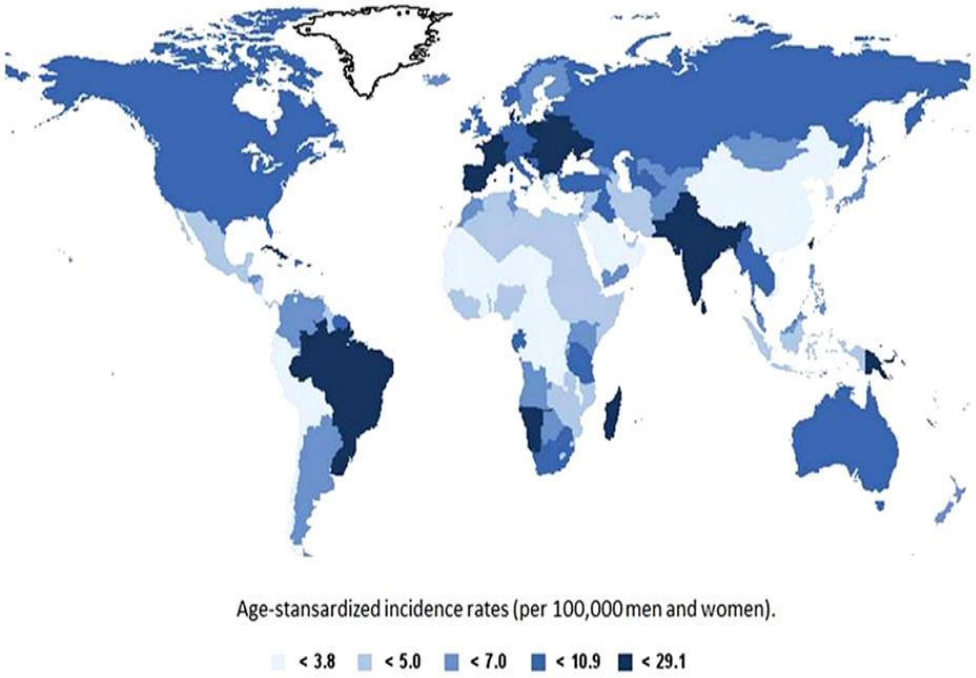

Figure 1.2: Age-standardized incidence rates (per 100,000 men and women) of head and neck cancer in the world. Incidence rates per geographic area are represented in different shades of blue per index. (Data source is IARC, GLOBOCAN).

GLOBOCAN 2012 report also revealed that pharyngeal cancer (apart from nasopharynx) accounted for 142,000 new cases (1% of the world total), and was much more frequent among males than among females (sex ratio of 4:1). The region with the highest incidence for both sexes was Western Europe (7.5 per 100,000 and 1.6 per 100,000 respectively), with the lowest rates (less than 1.0) in Northern and Western Africa and Western Asia. Globally, 96,000 deaths occurred due to pharyngeal cancers (1.2% of the world total).

Nasopharyngeal cancer (NPC) is a relatively uncommon tumour (87,000 new cases, 0.6% of all cancers), more frequent in males (sex ratio of 2.3:1) with very distinct geographic areas of high risk. The highest incidence rates are in populations of South-Eastern Asia (6.4 in men, 2.4 in women), and in Micronesia/Polynesia, Eastern Asia and Northern Africa, where rates are above two and one in men and women respectively. Around 51,000 deaths from NPC occurred, representing 0.6% of all cancer deaths (Ferlay et al. 2015).

1.2.2 Incidence in India

The actual numbers of head and neck cancer (HNC) cases in India are much higher than reflect through the accessible literature and hence can be considering as a 'tip of iceberg' situation. This situation further supported by the latest reports of 'Net-based Atlas of Cancer in India'. Regions including Western Asia and South East Asia would face sharp increases of over 75% cancer deaths in 2020 as compared to 2000. HNC is the commonest malignancy encountered in Indian males and oral cavity cancer is the most familiar type of HNC (Mishra and Meherotra 2014). The Indian Council of Medical Research (ICMR) initiated the National Cancer Registry Programme (NCRP) with a network of Cancer registries across the India. NCRP have three Population Based Cancer Registries (PBCRs) at Mumbai, Chennai and Bangalore, and three Hospital Based Cancer Registries (HBCRs) at Thiruvananthapuram, Chandigarh and Dibrugarh. The numbers of PBCRs gradually extended over the years and as of now, the network of NCRP has 23 PBCRs (*http://www.ncrpindia.org*).

The data accrued by NCRP reflects the trends in the age adjusted incidence rates for the different sites of head and neck cancers in Mumbai, Bangalore, Chennai, Kolkata, Delhi, Bhopal, Barshi, Chandigarh, Dibrugarh and Thiruvananthapuram, and nine Northeast Indian registries. According to recent report of NCRP on time trends, it is estimated that future burden of cancer cases in India might increase and will be 13,20,928 (male 6,22,203, female 6,98,725) in 2020 (Sharma et al. 2014). Head and neck cancers account for 30-40% cancers at all sites in India, out of which 9.4% are oral cancers (Bhattacharjee et al. 2006). An estimate of over 200,000 head and neck cancer occurs in India each year of which 80,000 were oral cancer. Around 40,000 and 29,000 cases of pharyngeal and laryngeal cancer occur in India every year respectively (Kulkarni 2013). According to NCRP database, different Indian regions including Bhopal (12.6% of all cancer cases), Barshi (11%), Mumbai (9.2%), Ahmadabad rural (13.9%), Nagpur (13%) Pune (10.2%) and Wardha (18%) have mouth cancer as a leading site of cancer in males. Based on accessible data, Bhopal has the world highest incidence rate of the oral cavity cancer in both males and females.

In Northeast India, head and neck cancer is one of the common cancers and incidence of oral cancers is around 33%. Population Based Cancer Registry (PBCR) Guwahati was formed in 2003 at Dr. B. Borooah Cancer Institute, Guwahati-Assam and its covers the entire area of Kamrup Urban (a major district in North East-India). Age Adjusted Rate (AAR) per 100,000 populations for all sites was calculated by NCRP for their report 2009-2011. In Kamrup, oesophagus cancer was the most common cancer in males, which comprised 14.47% of all cancer cases in male, and 10.42 % in females, followed by Hypopharynx in male (8.25%). According to the recent NCRP Report, among the residents of Cachar district of Assam, the leading sites of cancers in male were oesophagus (8.7% of all cancer cases and AAR=11.7), followed by lung (7.6% and AAR=10.5), hypopharynx (7.6%, and AAR=10.1), tongue (5.4% and AAR=7.5) and larynx (5.1% and AAR=6.5). In Dibrugarh, (another district of Assam) cancer of hypopharynx, mouth and tongue contributed 11.9%, 9% and 4.4% of total cancer cases in male respectively. In Nagaland, nasopharyngeal cancer was the most common cancer in male (19.3%) and comprised 10.9% in female. Whereas, Tripura and Mizoram, hypopharynx cancer contributed 7.2% and 4.7% of cancer cases in males. In Shillong, Meghalaya, oropharyngeal and oral cancer is the most familiar head and neck cancers. Oropharynx, oral, hypopharynx and larynx cancer constituted 24.2%, 23.9%, 18.4% and 15.6% of total head and neck cancer respectively in Meghalaya of Northeast India (Shunyu and Syiemlieh 2013).

1.3 Aaetiology of Head and Neck Cancer

The aaetiology of head and neck cancer is a complex, mixed up with a variety of environmental, toxic, and viral agent. The high incidence and mortality rates of head and neck cancer in Southeast Asia are due to lifestyle risk factors for example smoking, betel quid chewing and alcohol drinking (Baez 2008, Petti 2009), in addition, HPV infection and genetic factors also plays a major role in HNC (Chen et al. 2008).

1.3.1 Tobacco Smoking

Tobacco contains nicotine (psychoactive alkaloid), which bind to nicotinic acetylcholine receptors, cause increased heart rate, vasoconstriction, and alertness

(Petti 2009). Worldwide, smoking of tobacco in various forms is common practice**.** Smoking tobacco in the form of cigarettes and bidi is most common in India and in other South Asian countries. Tobacco smoke contains a complex mixture of about 5,000 chemicals and at least 60 of them are carcinogenic. The most important of them are nitrosamines, such as 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and N-nitrosonornicotine (NNN); polycyclic aromatic hydrocarbons (PAH), such as aromatic amines and benzo [α] pyrene. These carcinogens induce DNA adducts, mainly 6-methyl-guanine, which interfere with DNA replication and can damage cell proliferation and immune system. Derivatives of tobacco smoke induce upregulation of antiapoptotic factors, as well as activation of the transcription factor NF-κB, whose function is associated with autoimmune and neoplastic process. In addition, tobacco smoke induces reactive oxygen species (ROS), which activate pro-inflammatory genes (interleukin-8 and TNFα) expression and promote chronic inflammation. Tobacco smoke related epithelial cell damage and immune system alterations facilitate the infection by a variety of microbial pathogens, including HPV. Smoke derivatives also inhibit the production of β-defensin, an antimicrobial peptide secreted by gingival cells in the saliva, to reduce ciliary motility and to stimulate the hypersecretion of mucus. All these events by tobacco smoking contribute to the decrease in antimicrobial functions and increase the affinity to develop chronic infections (Petti 2009, Pezzuto et al. 2015).

1.3.2 Smokeless Tobacco and Betel Quid Chewing

Use of smokeless tobacco in a variety of forms, is common among both men and women. Smokeless tobacco products, such as tobacco flakes, whole leaf and *Zarda* are widespread in India and Bangladesh, *Nass/ Naswar* in Pakistan and other countries of Asia, and *Shamma* is common in Saudi Arabia. Other popular forms of smokeless tobacco are *Panmasala* and *Gutkha*, which are available since the introduction into the Indian market in 1980s. *Panmasala* and *Gutkha* are mixture of tobacco, areca nut, lime and catechu. The blend of ingredients is strongly genotoxic and carcinogenic (Nair et al. 2004).

The habit of betel quid chewing is very much common in many countries of Southeast Asia including India. There is an enormous diversity of variation in ingredients and ways of preparing betel quid, however, the most common

ingredients of betel quid are: glossy heart-shaped betel (*Piper betle*) leaves (envelop the other components forming a quid), the nut (Areca catechu), lime (calcium oxide), slaked lime (calcium hydroxide), or catechu (an extract of Acacia catechu with tannins and catechols). In addition, various flavour ingredients, such as spices and tobacco flakes are popular with betel quid (Gupta and Ray 2004, Petti 2009). Ingredients present in betel quid induce gene mutations and genotoxicity, resulting in damage to the salivary proteins, leading to changes in the structure of the oral mucosa, that initiate tumour development. (Merchant et al. 2000, Nair et al. 1985).

1.3.3 Alcohol Consumption

Chronic alcohol consumption is found to be associated with an increased risk for HNC (Petti 2009). Alcohol and its metabolites, mainly acetaldehyde, have numerous effects on the exposed cells including formation of ROS and cell cycle deregulation. The major alcohol metabolizing enzymes are alcohol dehydrogenase (ADH) that oxidises ethanol to acetaldehyde and has shown to be toxic, mutagenic and carcinogenic (Seitz and Stickel 2010). Moreover, Acetaldehyde hamper the DNA synthesis and repair machinery by inhibiting the enzyme o6-methyl-guanyltransferase, causes mutations in the hypoxanthine-guanine-phosphoribosyl transferase locus in human lymphocytes, induces sister chromatid exchanges, leading to gross chromosomal aberrations, inflammation and metaplasia in tracheal epithelium. Heavy alcohol consumption results in a complex alteration of both innate and acquired immune reaction. An excessive amount of alcohol intake induces a severe reduction in lymphocytes and natural killer cells, and that lytic activity of natural killers is impaired and not recoverable. (Molina et al. 2010, Yu et al. 2010).

1.3.4 Diet

The significance of diet and nutrition in head and neck carcinogenesis has been point out in some epidemiological studies (De Stefani et al. 1999, McLaughlin et al. 1988). Fruits and green vegetables that are rich in beta-carotene, Vitamins A, E and C are considered protective against oral cancer, whereas meat and red chilies are risk factors. Antioxidant and anti-carcinogenic properties of fruits and vegetables, such as Vitamins A, C and E, carotenoids, flavonoids, phytosterols, folates and

fibres, could play an vital role in counterbalancing the detrimental effects of carcinogen found in tobacco smoke, alcohol or in betel quid. However, the mechanisms of action and the relative roles of various micronutrients responsible for protection are not completely clear (Petti 2009). Dietary irons are necessary to maintain the epithelium thickness, whereas iron deficiency results in Plummer-Vinson syndrome and oral epithelial atrophy (Negri et al. 2000).

1.3.5 Human Papillomavirus (HPV) Infection

Human papillomaviruses (HPVs) are double stranded DNA viruses and comprises of a heterogeneous family of more than 130 different types (zur Hausen 2002). The HPV genome is composed of 7,200 – 8,000 DNA base pairs with a molecular weight of 5.2×10^6 Dalton. Based on base pair (bp) distribution, the viral DNA is divided into three parts: a 4,000 bp region that is responsible for DNA replication and cell transformation of virus; a 3,000 bp region that encodes the viral structural proteins and 1,000 bp non-coding region (NCR) that have the origin of viral DNA replication (Chaudhary et al. 2009). The HPV DNA has six early (E1, E2, E4, E5, E6, and E7), two late (L1 and L2) coding and one non-coding long control region (Ganguly and Parihar 2009). The E5, E6 and E7 genes are identified as oncogenic and they encode viral proteins. These proteins deregulate tumour suppressor function of p21, p53, and pRb proteins, resulting damage in apoptosis, DNA repair, cell cycle control system, and eventually leading to cancer development. The E5 protein stimulate cell growth through the activation and upregulation of the epidermal growth factor receptor (EGFR), thus initiating signaling cascades leading to the overexpression of proto-oncogenes and the suppression of cyclin dependent kinase inhibitor 1A (CDKN1A/p21) expression (Steger and Corbach 1997). The E6 oncoprotein (ubiquitin ligase) target the p53 tumour suppressor, thus promote p53-dependent cell-cycle arrest and apoptosis (Scheffner et al. 1990). On the other hand, the HPV E7 protein by ubiquitin-mediated degradation, deactivates the pRB tumour suppressor protein (Boyer et al. 1996). Deactivation of pRB by E7 causes release of E2F, (transcriptional regulator of cell proliferation) from pRb/E2F complexes, resulting in overexpression of the p16INK4a (a cyclin-dependent kinase inhibitor) (Khleif et al. 1996). Thus, detection of p16INK4a expression level considered as a biomarker for HPV infection. The E7

oncoprotein also exhibits kinase activity and form indirect complexes with cyclins A and E (McIntyre et al. 1996). Cyclins A and E are considered to play an important role in driving cellular proliferation. HPV-positive HNSCC is clinically, pathologically and aetiologically distinct with significantly improved prognosis as compared with HPV-negative HNSCC (Lewis et al. 2010, Ragin et al. 2006). The molecular mechanisms behind the favourable prognosis of HPV-related/p16 overexpressing HNSCC are not clearly evaluated (Bose et al. 2013). Moreover, deactivation of p53 and other tumour related genes through promoter hypermethylation has also been reported in HNSCC (Ha and Califano 2006). Two recent exome sequencing studies found significantly higher p53 mutations rates in HPV-negative HNSCC compared with HPV-positive tumours. (Agrawal et al. 2011, Stransky et al. 2011). It is now clear that HPV-positive and HPV-negative HNSCC are distinct subtype and classification of HNSCC by HPV status is crucial for the accurate and effective treatment and prognostication (Bose et al. 2013).

1.4 Clinical Perceptive of Head and Neck Cancer

Clinical diagnosis of head and neck cancer is performed by oral nasopharyngeal visual inspection, laryngoscopy or oesophagoscopy depending on the clinical symptoms. The imaging studies including a computerized tomography (CT) scan, positron emission tomography (PET) scan, magnetic resonance imaging (MRI), or combination of imaging techniques (e.g. PET-CT) which is more accurate than either method alone can be use for staging and assessment. For histopathological evaluation and classification of the lesion, a biopsy of the suspected lesion is the standard option. These analyses will be useful to assess patient prognosis and treatment (Argiris et al. 2008).

1.4.1 Sign and Symptoms

Head and neck cancers symptoms may include a sore throat that does not go away, unhealed lump or a sore, difficulty in swallowing, mouth ulcer and mouth bleeding, tongue pain and a change or hoarseness in the voice. It is essential to check with a physician about any of these symptoms. Breathing, hearing or speaking trouble, pain in the neck or the throat that does not leave, pain when swallowing, frequent headaches, pain or ringing in the ears may indicate pharyngeal cancer.

Whereas, ear pain or pain when swallowing may signifies laryngeal cancer. The common symptoms for paranasal sinuses and nasal cavity cancers are bleeding through the nose, chronic sinus infections that do not respond to treatment with antibiotics, swelling or other trouble with the eyes, frequent headaches, pain in the upper teeth or problems with dentures.

1.4.2 Staging

Tumours staging allows the doctor to assess prognosis and decide the proper treatment. Tumours are staged according to the size of the primary tumour (T), regional lymph node status (N), and the presence or absence of metastasis (M); these together are known as the TNM staging system [American Joint Committee on Cancer (AJCC)]. The assertion of the TNM system is that patients with smaller tumours and without nodal disease and distant metastases have a better prognosis than patients with larger tumours and/or presence of lymph node positivity and metastases (Patel and Shah 2005).

Basic TNM classification of cancer by clinicians

Definition of primary tumour (T)

TX – Primary tumour cannot be assessed
T0– Primary tumour is not evident
Tis– In situ carcinoma (pre-invasive)
T1 – Tumour that is 2cm or less in maximum dimension
T2 – Tumour that is more than 2cm but not more than 4cm in maximum dimension
T3 – Tumour that is more than 4cm
T4 – Tumour (larger than 4 cm) that extended up to muscle, bone, skin and anterior neck

T4 tumours further subcategories into T4a and T4b based on involvement of vital structures and thus their suitability for surgical resection.

T4a – locally advanced but resectable tumour
T4b – tumour that is not technically resectable but is suitable for nonsurgical options such as chemoradiotherapy

Definition of regional lymph node (N)

NX – Regional lymph nodes cannot be assessed

N0 – regional lymph node is not evident

N1 – movable homolateral lymph node in region that is ≤ 3cm in maximum dimension

N2a – movable homolateral lymph node in region that is ≤ 3-6cm

N2b – multiple homolateral lymph nodes in region that is ≤ 6cm

N2c – contralateral or bilateral lymph nodes is evident in region that is ≤ 6cm

N3 – metastasis in lymph node that is more than 6cm in maximum dimension

Definition of distant metastasis (M)

MX – Distant metastasis cannot be assessed

M0 – No distant metastasis is evident

M1 – Distant metastases is evident

Following are classification of head and neck cancer staging (Chen et al. 2008).

Stage I: T1 – N0 – M0

Stage II: T2 – N0 – M0

Stage III: T3 – N0 – M0; T3 – N1– M0

Stage IV A: T4a–N (0-2) – M0; T – N0 – M0; T(1-3) – N2 – M0

Stage IV B: T4b – any N – M0; any T – N3 – M0

Stage IV C: any T – any N – M1

Unfortunately head and neck cancers patients often present to their doctor with late stage tumours (e.g. III/IV), and therefore have a worse prognosis than early stage (e.g. I/II) patients.

1.4.3 Treatment

The most common HNSCC treatments are surgery and radiation, however, no universal standard mode of therapy exists. Surgery is particularly helpful for small resectable lesions in the oral cavity, pharynx and larynx. Other HNSCC treatments depend on the location of the tumour and presence of metastasis and include radiation, chemotherapy, chemoradiotherapy, and more recently, targeted

therapies. Radiotherapy (RT) may be used to reduce the size of the tumour before surgical resection; and is often used to eliminate any remaining cancer cells after surgical resection. RT is commonly used in conjunction with imaging technologies to allow for improved delivery of the tumour, mainly when imaging allows the 3D visualization of the tumour. RT is especially useful in treatment of laryngeal oropharyngeal and hypopharyngeal cancer as its cure rates are as successful as surgery, and has lower morbidity (Argiris et al. 2008). Chemotherapy was regularly used as a sedative treatment for HNSCC patients. Chemotherapy contains platinum compounds (e.g. cisplatin, carboplatin), taxanes and antimetabolites and have been used in advanced HNSCCs that are non-resectable and show high levels of recurrence. The chemoradiotherapy has been useful in unresectable late stage (III/IV) HNSCCs, shown improved result than radiation or chemotherapy alone. The use of radiotherapy and chemotherapy, or both, may have long-term side effects; thus, treatment may not only determine the outcome but also quality of life. A better understanding of HNSCC is thus required for future treatments (Argiris et al. 2008).

1.5 Genetic Alterations

Cancer is a multistep process caused by alterations in tumour-suppressor genes, oncogenes, and microRNA genes. These alterations are typically somatic events, although germ-line mutations can influence a person to heritable or familial cancer. A single genetic change can be not enough for the development of tumours. Many molecular level study demonstrated a multistep process of many sequential alterations of oncogenes, tumour-suppressor genes, or microRNA genes in malignant cell (Croce 2008). For better understanding of cancer aetiology, analysis of DNA sequence variants that contribute to cancer risk offers a good method. Variation of DNA sequence is termed as polymorphism if it is present at an allele frequency higher than 1% in the general population. Genetic polymorphisms are common all over the genome. The single nucleotide polymorphism (SNP) is the most common type of genetic polymorphism and occurs as frequently as one out of every 300 base pairs. Polymorphism may occur in introns and exons of genes and may have an impact on the structure and function of the protein, particularly when the polymorphism leads to an amino acid change in conserved domains. If the protein is involved in processes such as DNA repair, cell cycle control or

metabolism of toxic substances, alter in function of protein may be associated with a different susceptibility toward disease in variant allele carrier. The genetic polymorphisms of different cancer-related genes may be considered as biomarkers for cancer predisposition. Several studies support the hypothesis that genetic polymorphism plays an important role in HNSCC aetiology (Evans et al. 2004, McWilliams et al. 2000, Sturgis et al. 1999). Many studies on HNSCC susceptibility explored that not only the exposure to the potential carcinogens, but also other factors such as inter-individual genetic differences in the metabolism of tobacco smoke carcinogens, may play an important role in the progression of HNSCC.

1.5.1 Metabolic Genes Polymorphisms

Metabolic enzymes are responsible for metabolism of many exogenous chemicals that are toxic, mutagenic or carcinogenic. Genetic polymorphisms (mainly SNPs and deletions) of xenobiotic metabolizing gene affect the carcinogen metabolism. Xenobiotics (foreign substances) are hydrophobic in nature, enter through the lipid bilayers into cells, and require chemical activation before they interact with the cellular machinery, known as biotransformation. Xenobiotic metabolizing enzymes provide defence against carcinogenic activity, are divided into two main phases (Phase-II and I). Phase-I enzymes are responsible for oxidation, reduction or hydrolysis and encounter the xenobiotics and form functional groups and reactive centre on substrates. These reactive substrates are recognized by phase-II enzymes, which make them inactive and water soluble form through conjugation (Vineis 2002).

Phase-I oxidation reactions are carried out by cytochrome P450 (CYPs), epoxide hydrolases, and flavin-containing monooxygenases (FMO). The CYPs and FMOs are composed of superfamilies of enzymes and have multiple genes. The human cytochrome P450 family-1 memberA1 is encoded by the cytochrome P450 1A1 (*CYP1A1*) gene, is a member of the CYP superfamily and located in chromosome 15q22 to q24 and encodes aryl hydrocarbon hydroxylase (AHH), a phase-I metabolizing enzyme (Gonzalez 1988, Vondracek et al. 2001). The CYP1A1 enzymes can activate environmental carcinogens to convert them in to epoxide intermediates. Subsequently, the phase-II detoxification enzymes, such as glutathione S-transferases (GSTs) make these intermediates into water-soluble form

by conjugation with glutathione, so that carcinogenic substances easily excreted out from the human body and protect the body from toxic effects of carcinogens (Nebert and Dalton 2006, Soya et al. 2007). Four common polymorphisms have been identified in *CYP1A1*. The most widely studied polymorphism of *CYP1A1* is ***m1*** T3801C (rs4646903), which is characterized by T→C transition at 3801 nucleotide position in the 3′ non-coding region and that creates a *MspI* restriction enzyme cleavage site (Garte 1998, Zhou et al. 2009). Other common polymorphisms of *CYP1A1* includes: ***m2*** A2455G substitution leading to change of isoleucine to valine at codon 462, ***m3***- T3205C substitution in 3′ non-coding region and ***m4***- C2453A substitution leading to change of threonine to asparagine at codon 461. The *CYP1A1* ***m1*** T3801C polymorphism may affect level of messenger RNA (mRNA) stability or gene expression and thus may alter the enzyme activity (Shah et al. 2009). Therefore, polymorphisms of *CYP1A1* gene, which may cause enhanced enzymatic activity, become play a vital role in susceptibility to DNA adduct formation, leading to increased risk of cancer development.

On the other hand, the phase-II detoxifying enzymes facilitate the elimination of xenobiotic and the inactivation of electrophilic and potentially toxic metabolites produced by phase-I enzymes. The phase-II enzymes have received huge importance due to their role in detoxification of tobacco carcinogens such as PAH diol epoxides, aromatic amines, hydrazines and oxidative stress byproducts. The phase-II enzymes include several superfamilies of conjugating enzymes. Among the phase-II enzymes most important superfamilies of enzymes are the glutathione-S-transferases (*GST*), UDP-glucuronosyltransferases (*UGT*), sulfotransferases (*SULT*), *N*-acetyltransferases, and methyltransferases (*MT*) (Jancova et al. 2010). Glutathione S-transferases (GSTs) are important group of xenobiotics metabolizing enzymes involved in Phase-II detoxification of carcinogens. These enzymes play a vital role in the detoxification of many endogenous and exogenous substrates through conjugation of glutathione (a tripeptide consisting of glycine, glutamic acid, cysteine) to electrophilic compounds, resulting in less reactive and more easily removable glutathione conjugates. Among the three mammalian *GSTs*, (mitochondrial, cytosolic and microsomal) cytosolic *GSTs* characterize the largest family and shows significant genetic polymorphism. Cytosolic GST isoenzymes can be classified based on their substrate specificities,

isoelectric points and amino acid sequence homologies into major classes. The different isoenzyrnes of cytosolic GSTs are α (alpha), κ (Kappa), μ (Mu), ω (Omega), φ (Pi), π (Sigma), θ (Theta) and ζ (Zeta). Many studies have investigated the role of GST variations in modulating individual susceptibility to head and neck cancers. *GSTM1* and *GSTP1* plays significant role in the metabolization of PAH diol epoxides, whereas *GSTT1* contributes in the detoxification of potentially carcinogenic monohalomethanes and reactive epoxide metabolites of butadiene, all these carcinogens are constituent of tobacco smoke (Townsend and Tew 2003). Among the glutathione S-transferases (GSTs) superfamily, *GSTM1* and *GSTT1* genes were found to be associated with various cancers susceptibility (McIlwain et al. 2006). The *GSTM1* and *GSTT1* genes have been located in chromosome 1p13.3 and 22q11.2, respectively. *GSTM1* and *GSTT1* genes are polymorphic in nature, and frequent homozygous deletions of these genes resulting a loss of functional activity of corresponding enzymes and thus decreased detoxification capacity and promote cancer development (Mondal et al. 2013).

1.5.2 DNA Repair Genes Polymorphisms

The DNA repair system is one of the primary defence systems that eliminated the wide range of DNA damage, which is necessary for keeping the genomic integrity of the cells. Any alteration in the DNA repair pathway is likely to cause chromosomal instability, leading to cell malfunctioning and tumourigenesis (Horton et al. 2008, Paz-Elizur et al. 2008). An individual's capacity to repair DNA damage vary widely on exposure to tobacco smoke, smokeless tobacco, ultraviolet radiation and endogenous reactions that contain oxidants (McWilliams et al. 2008). Polymorphisms in DNA repair genes are very common, and several studies have demonstrated a significant association of repair genes polymorphisms with cancer risk (Auranen et al. 2005, Zienolddiny et al. 2006). The DNA repair genes involved in cancer are grouped into four major repair pathways: Base excision repair (BER), nucleotide excision repair (NER), mismatch repair (MMR) and double-strand DNA breaks repair (DSBR). *XRCC1* (X-ray repair cross-complementing group 1) gene is an important component of the BER pathway of DNA repair system. *XRCC1* gene encodes a protein that interact with other BER proteins such as DNA polymerase-beta (POLB), DNA ligase III (LIG3), and poly (ADP-ribose) polymerase (PARP) to

be involved in the competent DNA repair of single strand breaks (Dianova et al. 2004). The *XRCC1* gene having three common single nucleotide polymorphisms (SNPs) that lead to amino acids changes in codon 194 (C→T; exon 6; Arg to Trp), codon 280 (G→A; exon 9; Arg to His,) and codon 399 (G→A; exon 10; Arg to Gln,) respectively. These substitutions of amino acid may affect the repair of DNA single-strand breaks by altering the protein–protein interactions between XRCC1 and other BER proteins. *XRCC2* (X-ray repair cross-complementing group 2) gene encodes a protein that is involved in the homologous recombination repair (HRR) pathway of double-stranded DNA, thought to repair chromosomal fragmentation, translocations and deletions. In *XRCC2*, common polymorphic variant is amino acids substitutions in codon 188 (31479 G→A; exon 3; Arginine to Histidine). This polymorphic locus of *XRCC2* identified to be associated with various types of cancer (Jiao et al. 2008, Johnson et al. 1999). Polymorphisms of *XRCC2* genes are suppose to be a genetic modifier for tobacco-related cancers. In addition, the roles of gene–gene interaction and gene–environment interactions in cancer progression have increased important.

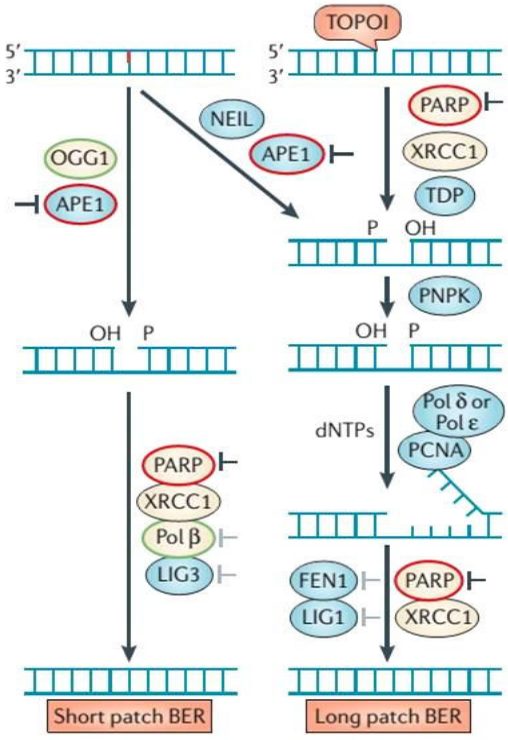

Figure 1.3: Base excision Repair (BER) pathway. XRCC1 along with PARP1 protein facilitate DNA repair by recruiting other repair proteins and providing the scaffold for a short patch and long patch BER pathway (Curtin 2012).

1.6 Epigenetic Alterations

One of fundamental questions regarding the diversity of phenotypes within a population is why monozygotic twins or cloned animals can have different phenotypes and disease susceptibility despite their identical DNA sequences and classic genetics unable to explain these phenomena. However, the concept of epigenetics offers a partial explanation of these phenomena. In 1939, C.H. Waddington introduced "the causal interactions between genes and their products, which bring the phenotype into being". Later on, the term epigenetics was described as the study of heritable changes in gene expression without any changes in the DNA sequences. Epigenetic gene patterns play a fundamental role in diverse biological development including embryonic changes, X-chromosome inactivation and genetic imprinting (Esteller 2008, Tsai and Baylin 2011). Unlike genetic changes, epigenetic alteration are reversible and the key processes involved in epigenetic regulation include DNA methylation, chromatin modification (covalent alteration in core histones), nucleosome positioning and post-translational gene expression regulation by noncoding RNAs. Epigenetic changes occur more often than genetic mutation may persevere for whole cell life and even for multiple generations. Disruptions of these epigenetic processes can cause aberrant gene expression and function, which may lead to initiation, development and progression of cancer (Sharma et al. 2010).

DNA methylation is a covalent modification of the DNA molecule itself in which a methyl group attached to the 5^{th} carbon of the cytosine ring of a CpG dinucleotide by the enzyme DNA methyltransferase (DNMT). There are mainly three DNMTs, viz. DNMT1, DNMT3a, and DNMT3b. DNMT3a and DNMT3b are de novo enzymes that target unmethylated CpG island to initiate methylation; whereas, DNMT1 maintain the existing methylation patterns. The alternation in DNA methylation was first identified epigenetic marker associated with cancer. These alterations include hypermethylation and hypomethylation (Feinberg and Vogelstein 1983a, b).

DNA hypermethylation is the gain of methylation at specific sites mainly in promoter CpG sites. CpG sites are widely distributed in CpG-rich regions of the genome known as CpG islands. These CpG islands are located upstream from the

promoter region of a gene at the 5′ end (Ng and Bird 1999). These CpG islands are approximately 500 base pairs in length, form more than 55% of the nucleotides, and present in the promoter regions of 40-50% of mammalian genes. There are around 45,000 CpG islands distributed in the human genome. Aberrant DNA methylation of CpG islands causes gene silencing and, plays a key mechanism for carcinogenesis. CpG-island promoter hypermethylation can affect genes mainly involved in the tumour suppressor's pathway, the DNA repair system, the metastasis-related pathway, the metabolism of carcinogens, cell-to-cell interaction, cell cycle, apoptosis, and angiogenesis. In tumour cells, mainly, global hypomethylation is escorted by hypermethylation of CpG islands promoter that usually remains unmethylated in normal cells. This special pattern of individual CpG-islands promoter hypermethylation of different tumour suppressor genes is observed in most types of human cancers (Baylin and Jones 2011). However, the mechanism of aberrant DNA methylation remains unclear. The hypermethylation pattern of gene is different for each type of cancers; for example, *BRCA1* (DNA repair gene) is found to be hypermethylated in breast and ovarian cancer, but not at other sites (Esteller et al. 2001).

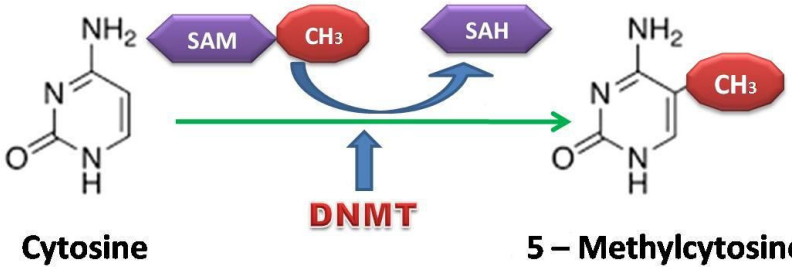

Figure 1.4: Schematic representation of DNA methylation. The transfer of a methyl group (CH_3) onto the 5-carbon position of the cytosine of CpG island to form a 5-methylcytosine by the action of DNA methyltransferease (DNMT).

DNA hypomethylation is the loss of DNA methylation in genome-wide regions. DNA hypomethylation was found in tumours was one of the first epigenetic alterations observed in human cancer (Feinberg and Vogelstein 1983a). DNA hypomethylation can assist mitotic recombination, leading to deletions and translocations, and it can promote chromosomal rearrangements. Three probable

mechanisms could explain the DNA hypomethylation in cancer development; these are: generation of chromosomal instability, reactivation of transposable elements, and loss of imprinting. The loss of methyl groups from DNA can also interrupt genomic imprinting, for example in mice models, with a loss of imprinting of IGF2 or overall defects in imprinting have an increased risk of cancer. In numerous cancer cells, promoter regions undergo demethylation and the normally repressed genes become expressed (Esteller 2008).

Another major epigenetic event in carcinogenesis is histone modification that altered chromatin structure, and plays an important role in gene regulation. The basic unit of chromatin is the nucleosome that consists of 146 base pairs of DNA surrounding a histone octomers of a highly ordered structure. These octamers contain double subunits of H2A, H2B, H3, and H4 core histone proteins. Histones can undergo multiple post-translational modifications by different enzymes such as histone acetyltransferase (HATs), histone methyltransferase (HMTs), histone deacetylase (HDACs and sirtuins) and histone demethylases (HDMs), kinases, phosphatases, ubiquitin ligases, deubiquitinases, sumoligases and proteases (Dillon 2006). Histones acetylation and methylation have direct effects on a variety of nuclear processes, such as gene transcription, DNA replication, DNA repair and the chromosomes rearrangement. However, effect of histone methylation depends on the type of amino acid and its position in the histone tail (Mack 2006). Usually, acetylation of histone is associated with transcriptional activation but de-acetylation of histone lead to repression for transcription and hence promotes gene silencing. The combination of both acetylation and histone modification is known as the 'histone code' and significant cross talk between the histone code and DNA methylation that together arbitrate gene silencing (Feinberg 2001). The combination of the hypo-acetylated and hypermethylated histones H3 and H4 can silences tumour-suppressor genes, despite the absence of hypermethylation of the CpG island (Esteller 2008). Expression patterns of histone-modifying enzymes differentiate tumour tissues from their normal counterparts, and they vary according to tumour types (Ozdag et al. 2006). The noncoding RNAs are also known to be played a crucial role in epigenetic alteration during cancer development. The small ncRNA include microRNA (miRNA), small interfering RNA (siRNA), small nucleolar RNA (snoRNA) and PIWI- interacting RNA (piRNA), and). Among these noncoding

RNAs, miRNAs (short, 22-nucleotide) are most extensively studied as they are very important to normal cell physiology, and alternation in their expression has been associated to several diseases, including cancer (Mitra et al. 2012, Sana et al. 2012). The miRNAs regulates gene expression by sequence-specific base pairing in the 3′ untranslated regions of the target mRNA. The miRNAs are transcribed by RNA polymerase-II, while miRNA synthesis, occur by RNA polymerase-III that are existing near to tRNA, Alu, and mammalian wide interspersed sequences (Macfarlane and Murphy 2010). Recent studies have explained that miRNA expression profile varies between normal tissues and tumour tissues and among tumour types (Chen 2005, Lu et al. 2005). DNA hypermethylation in the 5′ regulatory region of miRNA is a mechanism that can account for the down-regulation of miRNA in tumours. The methylation silencing of miR-124a causes activation of the cyclin D–kinase 6 oncogene (CDK6), and it is a common epigenetic events in tumours (Lujambio et al. 2007).

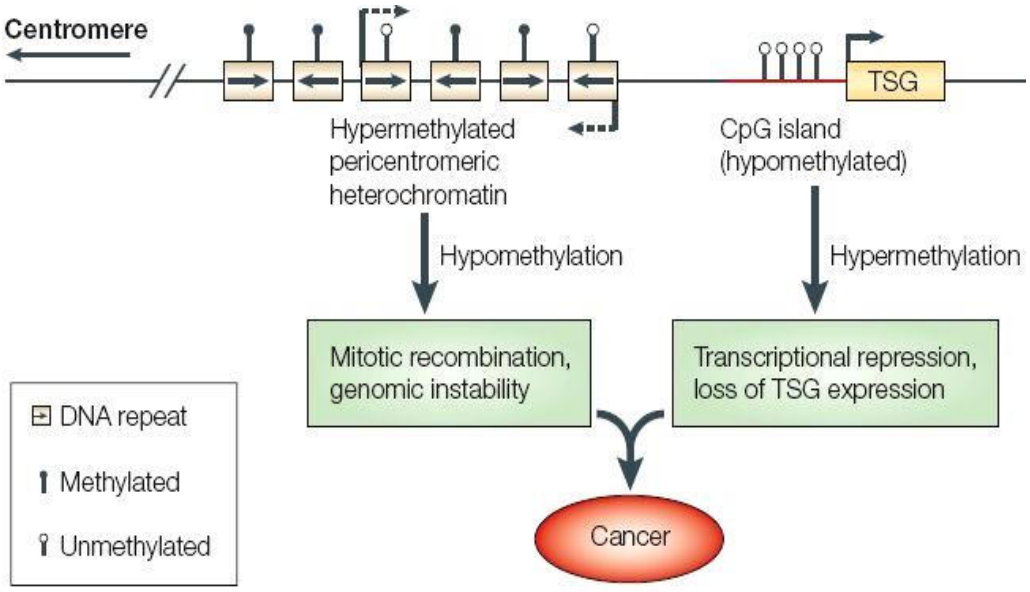

Figure 1.5: DNA methylation promotes events in tumourigenesis. This diagram shows the region contains DNA repeat, hypermethylated pericentromeric heterochromatin and activated tumour suppressor genes (TSGs) associated with a CpG island hypomethylated (indicated by red line) in normal cell. Whereas, in tumour cells, hypermethylated pericentromeric heterochromatin becomes hypomethylated, and this promotes genomic instability through increased mitotic recombination events. On the other hand, *de novo* hypermethylation of CpG islands of TSGs also occurs in cancer cells, and it can lead to the transcriptional silencing of tumour suppressing genes. These alternations of methylation are early events in tumourigenesis (Robertson 2005).

1.6.1 Promoter Methylation of Tumour Suppression Genes

Promoter hypermethylation of tumour-related genes are likely to be playing a vital in cancers. Many epigenetic alteration in various cancers have been studied recently and revealed the methods for detecting promoter methylation pattern of tumour-related genes to stratify high-risk groups (Dong et al. 2014, Laskar et al. 2014, Teodoridis et al. 2004). CpG islands hypermethylation in the promoter region of genes (those involved in cell cycle regulation, apoptosis, DNA repair and detoxification pathways) are associated with cancer development and progression (Baylin et al. 2001). Therefore, aberrant promoter methylation of CpG islands is part of the epigenetic alteration that will promise potential molecular biomarker for prediction and detection of head and neck cancers. Promoter hypermethylation and subsequent silencing of numerous tumours suppressor genes has been found in head and neck cancers (Hasegawa et al. 2002, Yalniz et al. 2011). Maximum emphasis has been given to the identification of genetic abnormalities that are conventionally thought to be the major molecular basis of tumour development in humans. Techniques like loss of heterozygosity (LOH) analysis, microsatellite instability (MSI) and identification of individual gene mutations have been widely used in the field of head and neck cancer (Boyle et al. 1993, Field et al. 1995). However, in recent years, many epigenetic studies had been done, which covered a broad group of tumour-associated pathway genes, including *p14*, *p15*, *p16* (cell cycle control); *DAPK*, *p73*, *RASSF1* (apoptosis); *BRCA1*, *MLH1*, *MSH2*, *MGMT* (DNA repair); *ATM*, *GSTP1* (carcinogen metabolism); *ECAD*, *CDH1*, *EDNRB* (cell-cell adhesion), and *MINTs* (methylated in tumours) loci such as *MINT1*, *MINT2* and *MINT31*. E-cadherinis (ECAD), a trans-membrane glycoprotein accountable for cell–cell adhesion and the altered expression of which is highly associated with regional metastasis in OSCC (Tanaka et al. 2003, Thomas and Speight 2001). The cell cycle regulatory genes (*p16*, *p15* and *p14*) have been extensively studied in head and neck cancer (El-Naggar et al. 1997, Huang et al. 2002, Ogi et al. 2002, Viswanathan et al. 2003). DAPK (Death associated protein kinase) is associated with loss of apoptosis and cell immortality, and its reduced expression has been associated with metastasis in different cancers. In head and neck cancer 27% of *DAPK* promoter hypermethylation has been observed (Sanchez-Cespedes et al. 2000). Recent studies also reported that *DAPK* and *p16* aberrant hypermethylation was associated with

poor prognosis in oral cancers (Su et al. 2010, Supic et al. 2011). Another frequently studied hypermethylated gene is *RASSF1A*, which belong to the Ras association family (RASSF) of proteins involved in the Ras/PI3K/AKT pathways. The MINT (methylated loci in tumours) family CpG islands are associated with tumours at several sites (Toyota et al. 1999b); however, their functions are indecisive; as they are not situated near to any known genes. Many studies found aberrant promoter methylation of *MINT1, MINT2* and *MINT31* in various cancer (Laskar et al. 2014, Ogi et al. 2002). Different methods have been used for methylation analysis, such as bisulfate sequencing, combined bisulfite restriction analysis (COBRA), methylation specific PCR (MSP), real time MSP or MethyLight, pyrosequencing, MassArray. All these methods primarily are based on principles that differentially recognize 5-methylcytosine (C^m) from cytosine (C). Bisulfite treatment of genomic DNA modifies or converts unmethylated cytosine (C) into uracil (U), whereas leaving methylated cytosine (C^m) unchanged. The most common and simple method of methylation study is methylation specific PCR (MSP). Flexibility of MSP in selecting a genomic segment is very large because primers of PCR can be designed at any positions.

1.6.2 CpG Island Methylator Phenotype (CIMP)

CpG island methylator phenotype (CIMP) related tumours are a different group defined by CpG–rich promoter hypermethylation in multiple genes and comprise of distinct epidemiological and molecular characteristics (Laskar et al. 2014). The concept of CIMP panel was first studied in colorectal cancer and demonstrated that colorectal cancer could be classified into two groups based on CIMP status: one group that shows rare or null methylation referred as CIMP negative, and the other group that shows aberrant DNA methylation of multiple genes simultaneously and referred as CIMP positive tumours (Toyota et al. 1999a). Later the CIMP panels of multiple genes were also investigated in many other cancers. This CIMP panel study open up a new pathway for cancer, besides to the mutation, microsatellite instable (MSI) and chromosomal instable (CIN) categories, ranking for a subset of cancer bearing excessive cancer-specific promoter region hypermethylation (Carmona and Esteller 2010). The classic CIMP panel contains five genes include p16, *MINT1*, *MINT2*, *MINT31* and *MLH1*, which provided an

easy and representative approach to define CIMP panel (Park et al. 2003). However, a new CIMP panel for colorectal cancer was suggested, which consist of *CACNA1G*, *IGF2*, *NEUROG1*, *RUNX3*, and *SOCS1* (Weisenberger et al. 2006). Further, CIMP panel has been extended, which include *CACNA1G*, *CRABP1*, *IGF2*, *NEUROG1*, *RUNX3* , *SOCS1* , *HIC1* , *IGFBP3* and *WRN* genes (Bardhan and Liu 2013). The 'right' definition of CIMP is still a highly controversial, due to the lack of an ideal gene panel and the suitable method of methylation detection. CIMPs have been reported for a number of cancers, including oesophageal squamous cell carcinoma (ESCC) (Ling et al. 2011), oral cancer (Jithesh et al. 2013), upper aerodigestive tract (UADT) (Talukdar et al. 2014) and breast cancer (Roessler et al. 2015) and HNSCC (Lechner et al. 2013). However, the probable role of CIMP pathway in HNSCC tumourigenesis is still not known.

1.7 Rationale of the Propose Study

Head and neck cancer (HNC) is one most common cancer and accounts for more than 650,000 new cases each year worldwide (Argiris et al. 2008). HNC accounts for the highest percentage of the cancer in Northeast (NE) India with oral cancer being the most common. In addition, morbidity and mortality of HNC in this part of India is very high (Bhattacharjee et al. 2006). Many previous studies have been done in NE Indian population to explore the association between risk factors and cancer (Chatterjee et al. 2009, Mondal and Ghosh 2013, Talukdar et al. 2013, Yadav et al. 2010). Tobacco smoking (cigarette/bidi), betel quid chewing (with or without tobacco) and alcohol consumption are common practice in NE India. Both smoke and smokeless tobacco contains a complex form of carcinogenic compounds; and generate reactive oxygen species (ROS) which can damage DNA. The causal role of high-risk HPV in the carcinogenesis of head and neck squamous cell carcinoma (HNSCC) has been investigated in recent studies. Especially in oropharyngeal squamous cell carcinoma (OPSCC). In addition, HPV-positive HNSCC is associated with a more favourable survival (Ramshankar and Krishnamurthy 2013).

The phase I and II metabolic enzymes activate and detoxify the carcinogens of tobacco product and excreted out from the body. However, inter-individual genetic difference may alter the activity of these and subsequently decrease

metabolizing capacity, thereby increasing susceptibility to cancer risk. On the other hand, DNA damage is fixed by the DNA repair systems. Any defect in the DNA repair pathway will lead to genomic instability, thus play a major role in tumourigenesis. Many previous studies investigated the association of polymorphisms in the carcinogen metabolic (*CYP1A1, GSTT1 & GSTM1*) genes (Biselli et al. 2006, Chatterjee et al. 2009, Hashibe et al. 2003) and DNA repair (*XRCC1 & XRCC2*) genes (Benhamou et al. 2004, Lou et al. 2013) in HNC but have generated inconsistent results. Furthermore, many of these studies were lacking for the interaction of these genes and the environmental factors. Identification of genetic susceptibility along with tobacco consumption status of the population will guide toward better understanding of HNC aetiology. We, therefore, performed a case-control study in the NE Indian population, to test the hypothesis that combined effects of tobacco carcinogens-metabolizing and DNA repair genes and their interaction with tobacco smoking and chewing might be associated with increased the risk of HNC. Moreover, we also included Multifactor Dimensionality Reduction (MDR) approach to predict high-risk and low-risk gene-gene and gene-environment interaction models that confer HNC risk.

Promoter hypermethylation profile of tumour-related genes was anticipated to be crucial and common in different cancers. Many epigenetic studies had found an association between CpG island promoter methylation of tumour suppression genes and cancers (Sanchez-Cespedes et al. 2000, Sharma et al. 2010, Shaw et al. 2006). Therefore, it is crucial to study the promoter methylation status of a panel of representative genes in HNSCC. There were no such studies conducted, focusing on specific promoter methylation profile in HNSCC with a combination of genetic risk factors related to xenobiotic and DNA repair pathway. Thus, variation in promoter hypermethylation pattern of HNSCC based on habits, genetic alteration and HPV infection is still unclear. To understand the underlying mechanisms of differential patterns of tumour-specific hypermethylation, we analyzed the aberrant promoter methylation profile of HNSCC using seven important tumour-related pathway genes (*p16*, *DAPK*, *GSTP1*, *ECAD*, *RASSF1, MLH1* and *BRCA1*) and three methylated loci (*MINT1*, *MINT2* and *MINT31*) in the high cancer incidence zone of NE India. We are the first to explore the correlation of CIMP characteristics with genetic (polymorphisms of *GSTM1*, *GSTT1*, *CYP1A1*, *XRCC1* and *XRCC2* genes) and

environmental factors (smoking, betel quid and tobacco chewing) and with HPV and survival status of HNSCC patients.

As per our knowledge, this is a first kind of study where we performed profiling of genetic alternation (genetic polymorphism of *GSTM1, GSTT1, CYP1A1, XRCC1*, *XRCC2*), epigenetic alternation (promoter hypermethylation of 10 tumour-related gene/loci), their combined effect (gene-gene interaction and gene-environment interaction) and the survival status in head and neck cancer from Northeast India.

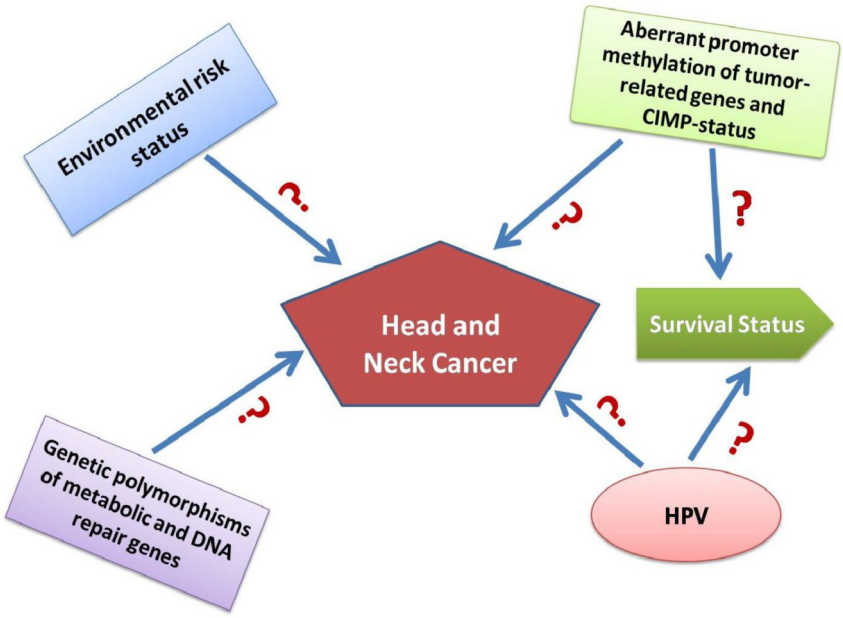

Figure 1.6: Diagrammatic representation of proposed study from Northeast India

1.8 Objectives of the Study

1. To study the human papillomavirus (HPV) prevalence in head and neck cancer and the association with survival status.

2. To study the genetic polymorphisms of metabolizing *(GSTM1*, *GSTT1* and *CYP1A1*) and DNA repair *(XRCC1* and *XRCC2*) genes in head and neck cancer, along with gene-gene and gene-environmental interactions.

3. To study the aberrant promoter methylation of tumour-related genes/loci (*p16*, *BRCA1*, *GSTP1*, *ECAD*, *RASSAF1*, *DAPK1, MLH1*, *MINT1*, *MINT2* and *MINT31*) and CpG island methylator phenotype (CIMP) status in head and neck cancer.

4. To study the correlation of promoter methylation profile of head and neck cancer with HPV, survival, environmental and genetic factors.