2.1 History of cancer

The oldest description of cancer appeared in several papyri dates back to ancient Egypt and was deciphered late in the 19th century [1]. The word cancer came from the Greek word "**karkinos" (**to describe tumors) and was termed by the physician Hippocrates (**460–370 B.C**), but he was not the first to discover this disease [1, 2]. Some of the earliest evidence of human bone cancer was found in human mummies of ancient Egypt; the bony skull destruction was seen as an evidence of cancer in the head and neck region. The world's oldest recorded cases, dating back to about 1600 BC have been found in the ancient manuscripts. Oldest recorded case of breast cancer in human history hails from ancient Egypt in **1500 BC** and it was recorded that there was no treatment for cancer, but only palliative treatment exist [3, 4].

In 1775, surgeon Percivall Pott, was the first surgeon to identify the cause of cancer for the first time and discovered that cancer of the scrotum was a common disease among chimney sweepers. In the 18th century English surgeon Campbell De Morgan discovered that 'cancer poison' eventually spreads from the primary tumor through the lymph nodes to other sites (metastasis). In the 19th century, Asepsis improved surgical sanitization and as the survival statistics went up, surgical removal of the tumor became the primary treatment for cancer [1].

Oral cancer was termed in the Sushruta Samhita, a treatise on Indian surgery written in Sanskrit around 600 B.C in the form of Vedas, the oldest sacred books of the Hindu religion. Sushruta was one of the earliest surgeon of the world recorded history [5]. Scripts in Ayurveda reveal that cancer was recognized as a set of diverse diseases, as early as 2,000-2,500 years ago. Most commonly occurring neoplasm were of oral cavity, nasopharynx, penis, rectum and esophagus. Cervical, bone and breast lesions were not mentioned by the early Ayurveda writers. In South India and South Asia oropharynx was found to be most frequent site of neoplasm, because of betel nut chewing, tobacco chewing, and smoking habits [6].

2.2 Cancer epidemiology

2.2.1 International scenario

Cancer continues to be a major health problem even with the advances in diagnosis and

treatment. In developed countries, it is the second most common cause of death after cardiovascular diseases [7]. Global action is required to stop the increasing burden of non-communicable diseases, especially in low-income and middle-income countries [7]. According to the 2012 WHO survey, there were 14.1 million new cancer cases worldwide, of which 8 million occurred in developing countries. Lung, breast, colorectal and stomach cancers accounted for more than 40% of all cases diagnosed worldwide. In men, lung cancer was the most common cancer (16.7% of all new cases in men). Breast cancer was by far the most common cancer diagnosed in women (25.2% of all new cases in women) and tumor related deaths among women has risen from third to first place [8].

In 2012, cancer-related deaths were estimated to be 8.2 million (about 22,000 cancer deaths per day) out of which 5.3 million was from developing countries. More than half of all cancer deaths each year are due to lung, stomach, liver, colorectal and female breast cancers [8, 9].

The estimate showed that 32.5 million people diagnosed with cancer within five years were alive at the end of 2012. Most were women with breast cancer (6.3 million), men with prostate cancer (3.9 million), and men and women with colorectal cancer (3.5 million) [8, 10, 11]. WHO survey also predicted that the burden of cancer will increase to 23.6 million new cases by 2030 [8].

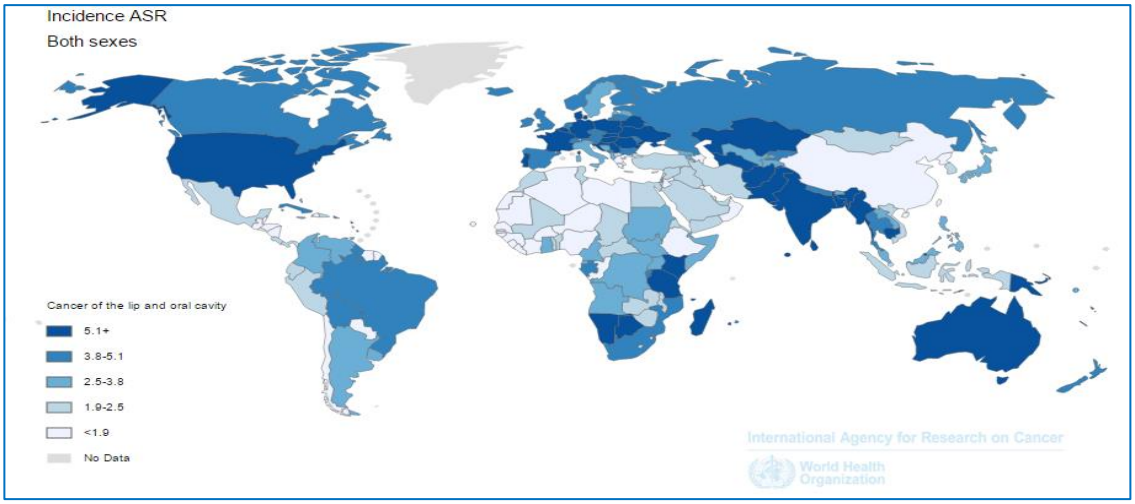

Figure 1: World map showing the distribution of age standardized rate of cancer incidence (Source: Globocan 2012, http://globocan.iarc.fr/Default.aspx) [11].

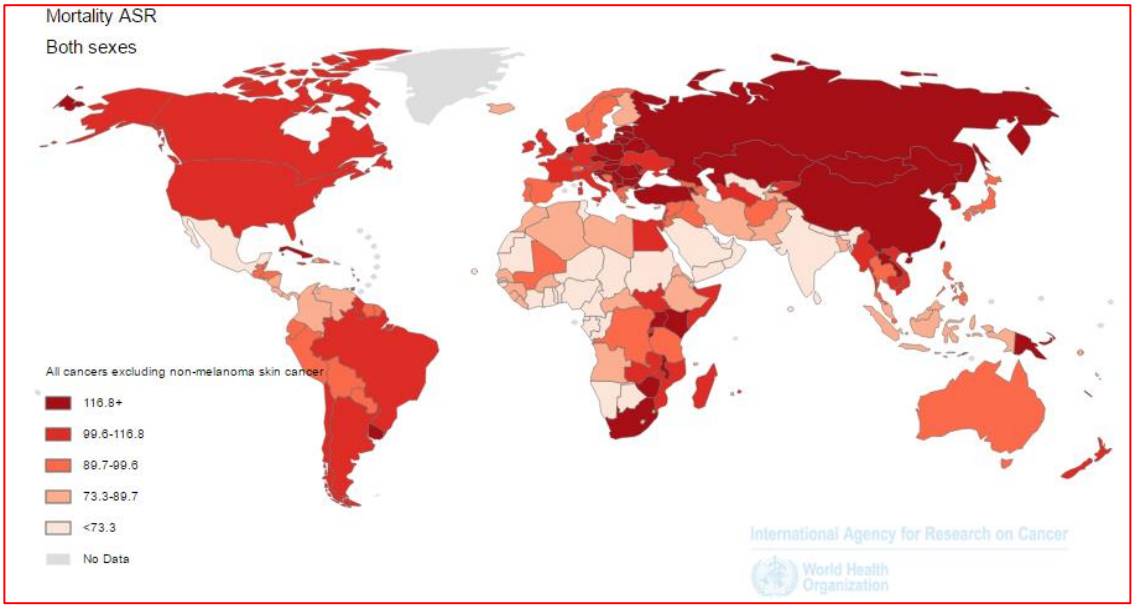

Figure 2: World map showing the distribution of age standardized rate of cancer mortality (Source: Globocan 2012, http://globocan.iarc.fr/Default.aspx) [11].

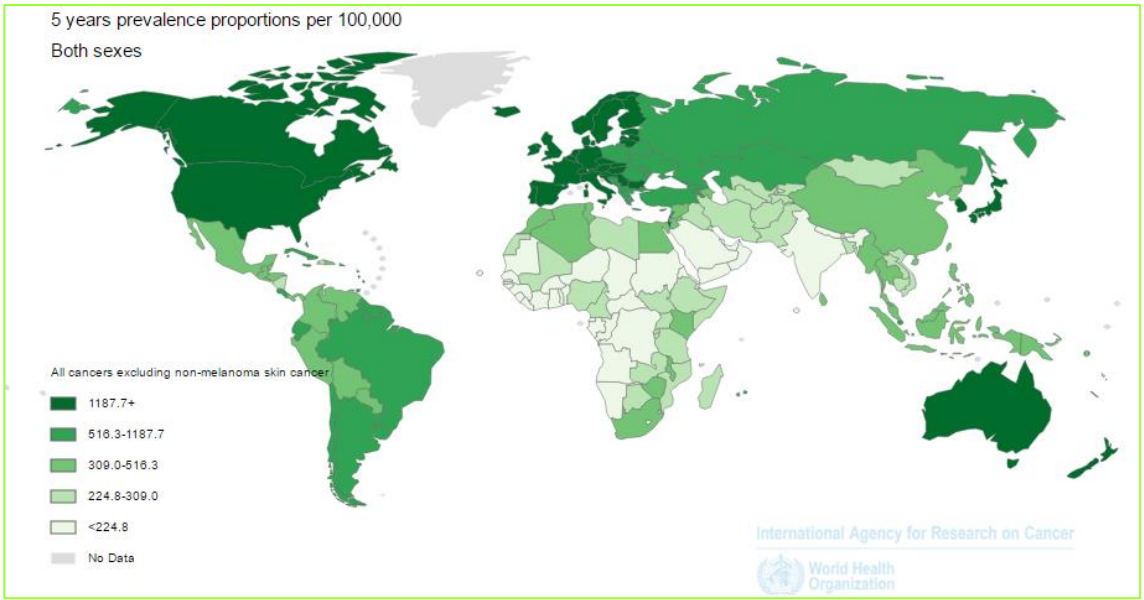

Figure 3: World map showing the distribution of prevalence of cancer (5-year, proportions per 100,000) (Source: Globocan 2012, http://globocan.iarc.fr/Default.aspx [11].

2.2.2 Indian scenario

In India, cancer has been one of the second leading cause of death after heart disease [13]. The total burden of cancer in India is estimated to be 1.0 million (94.0 per 100,000 incidences), 1.8 million (202.9 per 100,000) people living with cancer (within 5 year diagnosis) and 0.7 million (64.5 per 100,000) cancer deaths according to a survey by GLOBOCON in 2012. Cancer of the breast, cervix, lip and oral, lung and colorectum are most prevalent among all cancers in India [12].

Table 1: Estimated incidence, mortality and 5-year prevalence of top five cancer: both sexes [11].

Cancer	**Incidence**			**Mortality**			**5-year prevalence**		
	Number	%	ASR (W)	Number	%	ASR (W)	Number	%	Prop.
Breast	0.144	14.3	25.8	0.07	10.3	12.7	396991	22.2	92.6
Cervical	122844	12.1	22.0	67477	9.9	12.4	308901	17.3	72.0
Lip,Oral cavity	77003	7.6	7.2	52067	7.6	4.9	118902	6.6	13.5
Lung	70275	6.9	6.9	63759	9.3	6.3	32464	1.8	3.7
Colorectum	64332	6.3	6.1	48603	7.1	4.6	86650	4.8	9.8

Table 2: Estimated incidence, mortality and 5-year prevalence of top five cancer: men [11].

Cancer	**Incidence**			**Mortality**			**5-year prevalence**		
	Number	%	ASR (W)	Number	%	ASR (W)	Number	%	Prop.
Lip, oral cavity	53842	11.3	10.1	36436	10.2	6.7	83708	12.6	18.5
Lung	53728	11.3	11.0	48697	13.7	9.9	24473	3.7	5.4
Stomach	43386	9.1	8.6	40721	11.4	8.0	31050	4.7	6.8
Colorectum	36917	7.7	7.2	27814	7.8	5.4	49861	7.5	11.0
Other pharynx	31735	6.6	6.3	27002	7.6	5.3	46748	7.0	10.3

The five most frequent cancers (ranking defined by total number of cases) among men are of lip and oral cavity (11.3%), lung - 53728 (11.3%), stomach - 43386 (9.1%), colorectum - 36917 (7.7%) and pharynx - 31735 (6.6%) with age standardized rate of 10.1, 11.0, 8.6, 7.2 and 6.3 per 100,000, respectively. Cancer related mortality is highest in case of lung - 48697 (13.7%), followed by stomach 40721 (11.4%), lip, oral cavity 36436 (10.2%), colorectum 278114 (7.8%) and pharynx 27002 (7.6%) with age standardized rate of 9.9, 8.0, 6.7, 5.4 and 5.3 per 100,000, respectively. The 5 year

prevalence rate is higher in case of lip, oral cavity 83708 (12.6), followed by prostate 63818 (9.6%), colorectum 49861 (7.5%), pharynx 46748 (7.0%) and larynx 45444 (6.8%) cancer with age standardized rate 18.5, 14.1, 11.0, 10.3 and 10.0 per 100,000, respectively [11].

Table 3: Estimated incidence, mortality and 5-year prevalence of top five cancer: Women [11].

Cancer	**Incidence**			**Mortality**			**5-year prevalence**		
	Number	%	ASR(W)	Number	%	ASR(W)	Number	%	Prop.
Breast	144937	27.0	25.8	70218	21.5	12.7	396991	35.3	92.6
Cervix uteri	122844	22.9	22.0	67477	20.7	12.4	308901	27.4	72.0
Colorectum	27415	5.1	5.1	20789	6.4	3.8	36789	3.3	8.6
Ovary	26834	5.0	4.9	19549	6.0	3.6	55231	4.9	12.9
Lip, oral cavity	23161	4.3	4.3	15631	4.8	3.0	35194	3.1	8.2

In women the five most common cancers in India are breast 144937 (27.0%), cervix uteri 122844 (22.9%), colorectum 27415 (5.1%), ovary 26834 (5.0%) and lip, oral cavity 2316(4.3%) with age standardized rate of 25.8, 22.0, 5.1, 4.9 and 5.3 per 100,000, respectively. Cancer related mortality is highest in case of breast cancer 70218 (21.5%), followed by cervix-uteri (20.7%), colorectum (6.4%), and stomach cancer (5.6%). However, in terms of 5 year prevalence, the top most ranked is breast (35.3%), cervix-uteri (27.4%), ovary (4.9 %), thyroid (3.6 %) and colorectum (3.3%) cancer with age standardized rate of 92.6, 72.0, 12.9, 9.3 and 8.6, respectively. It has been predicted that around 1.08 million people will be diagnosed with cancer and 1.08 million will die due to cancer in 2030. The prediction also indicates that there will be around 0.23 million new cases and 0.16 million deaths due to head and neck (HNC) cancer (pharynx, nasopharynx, larynx and lip, oral cavity) in 2030 [11].

2.2.3 North-East Indian scenario

Recently, a report released by Population Based Cancer Registries (PBCR) of North Eastern (NE) region of India revealed that the total number of cancer cases registered was 0.3 million in the NE region in the year 2012-2014 [12]. According to Indian Council of Medical Research (ICMR) data on site specific cancer burden, cancer of

nasopharynx (NPC) has been found to be highest in the country, of which 55% of total NPC cases are reported from Nagaland, followed by Manipur (22.1%). In Assam and Mizoram the most prevalent cancers are esophageal and stomach cancer respectively (11.2% of all cancers in NE region) [13, 14]. In male, East-Khasi Hills district of Meghalaya had the highest Age Adjusted Rate (AARs) per 1000,000 in cancer of the tongue and hypopharynx, whereas Kamrup Urban District showed the highest AAR in cancer of the hypopharynx. Cancer associated with the use of tobacco was found in East-Khasi Hills district of Meghalaya with the highest related proportion of 69.5% and 45.0% for males and females, respectively [13].

Table 4: Number of Incident and Mortality Cases and Mortality-Incidence Percent (M/I %) [13]**.**

Registry (2012-2014)	**Male**			**Female**			**Male**	**Female**
	Incidence	Mortality	M/I%	Incidence	Mortality	M/I%	Total Deaths (TD)	
Kamrup Urban District, Assam state	3071	1011	32.9	2392	523	21.9	1011	523
Dibrugarh District, Assam	1498	433	28.9	1345	252	18.7	433	252
CacharDistrict, Assam	2666	412	15.5	2100	275	13.1	412	275
Manipur State	2081	560	26.9	2542	495	19.5	560	495
Mizoram State	2567	1346	52.4	2089	830	39.7	1346	830
Sikkim State	707	365	51.6	678	311	45.9	365	311
Meghalaya	2632	1027	39.0	1616	591	36.6	1027	591
Tripura State	3628	1778	49.0	2702	1082	40.0	1078	1082

In the Kamrup urban district (KUD), the leading sites of cancer in males were: oesophagus (14.3%), hypopharynx (8.9%), lung (8.4%), stomach (6.7%) and mouth (5.2%). The respective AAR per 100,000 populations for the sites was: oesophagus (29.1), hypopharynx (17.5), lung (18.7), stomach (14.1) and mouth (10.5). Among females the leading sites of cancer were: breast (17.5%), oesophagus (10.2%), gall bladder (9.3%), cervix-uteri (8.6%) and ovary (5.5%). The respective AAR per

100,000 populations for the sites was: breast (27.1), oesophagus (20.7), gall bladder (17.1), cervix uteri (14.5) and ovary (8.7) [13].

In Dibrugarh district, the leading sites of cancer in males were: oesophagus (15.3%), hypopharynx (11.6%), stomach (7.9%), mouth (6.8%) and lung (5.1%). The respective AAR per 100,000 populations for the sites was: oesophagus (14.6), hypopharynx (10.9), stomach (7.6), mouth (6.3) and lung (5.0). Among females the leading sites were: breast (19.0%), gall bladder (10.7%), oesophagus (9.4%), ovary (8.9%) and cervix-uteri (6.4%). The respective AAR per 100,000 populations for the sites was: breast (13.9), gall bladder (8.6), oesophagus (8.1), ovary (6.5) and cervix-uteri (4.9) [13].

Among Cachar district males, the leading sites were: oesophagus (10.2%), hypopharynx (8.6%), lung (8.4%), mouth (5.7%) and larynx (5.4%). The respective AAR per 100,000 populations for the sites was: oesophagus (12.9), hypopharynx (11.1), lung (11.5), mouth (7.1) and larynx (6.9). Among females, leading sites were: breast (14.3%) followed by cervix uteri (13.9%), gall bladder (10.3%), oesophagus (6.8%) and ovary (5.1%). The respective AAR per 100,000 populations for the sites was: breast (12.8), cervix uteri (12.7), gall bladder (10.2), oesophagus (7.1) and ovary (4.1) [13].

The incidence of head and neck cancer is alarmingly increasing in developing countries, and in NE India, the prevalence is found to be significantly high at 54.48%, affecting males more than females in the age group of 40–69 years [15]. In North-Eastern India, HNC incidence (~33%) is the highest among all the states. The etiological factor associated with HNC in this region is mainly due to the consumption of betel nut, tobacco in various forms, and lack of awareness. Furthermore, smoking, alcohol use, smokeless tobacco products are the major risk factors for oral cavity cancer, with smoking and alcohol having synergistic effects [16]. The survey of PBCR (2016) revealed that the burden of cancer will increase to estimate 1.73 million new cases and tobacco related cancer will be 0.53 million in NE population of India by 2030 [13].

2.3 Head and Neck Carcinoma (HNC)

Head and neck cancer (HNC) includes a broad spectrum of anatomical sites, from the upper aerodigestive tract including the lip, oral cavity (mouth), nasal cavity (inside the nose), paranasal sinuses, pharynx, and larynx [17, 18]. Though vast majority of the HNC cases are biologically similar, histologically, they varies from cases to cases. Most of the cases are either poorly or moderately differentiated and have the propensity to cause lymph node metastasis [18]. About 90% of the HNC cases are squamous cell carcinoma and rest 10% include verrucous carcinoma (VC), papillary squamous cell carcinoma (SCC), spindle cell squamous carcinoma, basaloid SCC, nasopharyngeal carcinoma, and adenoid SCC carcinoma [17, 18]. These SCC commonly originate from mucosal lining (epithelium) of these region [19].

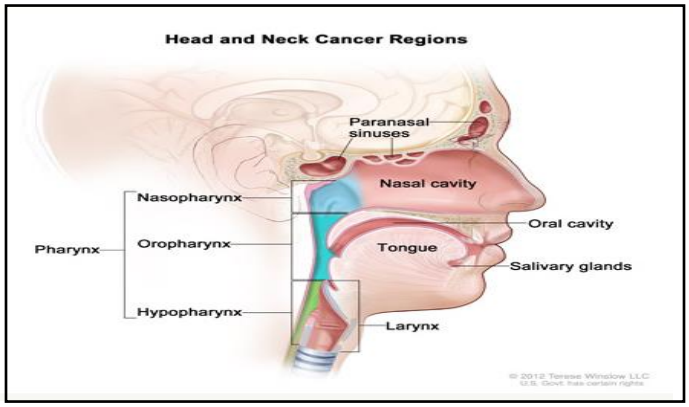

Figure 4: Illustration of different HNC region.

Figure consideration of squamous cell carcinoma commonly originates from mucosal lining (epithelium) of these regions [19]. Source: (*http://www.cancer.gov/types/head- and-neck/head-neck-fact-sheet*)

At least 75% of HNC are caused by tobacco and alcohol use; and other causal agents are UV light, particular chemicals and certain strain of viruses such as human papillomavirus (HPV) that increase the risk of several types of head and neck cancer [19, 20, 21]. HNC is highly curable if detected early, usually through surgery, radiation therapy or chemotherapy. The major issue of the HNC has indefinite symptoms and diagnosed only at the later stages which makes the treatment challenging worldwide [19, 22].

2.4 Risk factors related to HNC

Many epidemiological studies have attributed that betel-quid (BQ) chewing, tobacco chewing, cigarette smoking and alcohol are the crucial risk factors for HNC [23]. Betel nut chewing is a contributing factor to the development of HNC and esophageal cancer in Taiwan [24], and India [25, 26], and the anatomical sites of these two cancers are very closely related; oral habits (chewing, smoking and alcohol consumption) have a significant effect on the transformation of their normal epithelium to a malignant one. There are various types of carcinogen which are associated with different positions of risk in the development of head and neck squamous cell carcinoma (HNSCC).

2.4.1 Betel nut

The betel nut (BN), a fruit of the oriental palm (*Areca catechu*), is also called 'areca nut' in English, 'supari' in Hindi, 'shupari' in Bengali, 'tamul' in Assamese, 'adakka' in Malayalam, 'pakku' in Tamil [27]. The BN is most commonly used psychoactive substance, involving 600 million people around the world [28].

The habit of betel quid chewing (betel leaf, areca nut and lime) in India dates back since 2,000 years ago. Tobacco was introduced around the sixteenth century. It is expected that areca nut is consumed by at least 200 million individuals worldwide. The habit is prevalent mostly in South-East Asia and South Pacific islands. The betel quid chewing habit is in fact found all over the world wherever South East Asians particularly Indians have settled [29].

In Indian socio-cultural scenario, chewing of areca nut and betel quid is practiced for social acceptability and popularity, religious beliefs, perceived health benefits and addiction. In Hinduism betel leaf is regarded as an auspicious ingredient. Among the followers of the Hindu religion, areca nut (Supari) is considered as a vital ingredient in the offerings for God (Bhagwan) [30]. In Assam, betel nut, commonly known as 'tamul', is consumed as part of religious and cultural traditions, and health benefit beliefs prevalent among many indigenous people. Betel nut/tamul is very commonly offered at important social gatherings in this region.

However, besides the religious connections, areca nut is regarded by many people in South Asia as good for health, and it is used as a traditional medicine. It is used as mouth freshener after meals, a taste enhancer, purgative and intoxicant. It is also regarded beneficial for indigestion, impotence, gynecological problems and parasitic intestinal infection [30].

The basic component of stimulant chew is used in combination with betel nut/tamul betel leaf and slaked lime, with tobacco (zarda, gutkha, khaini) or without tobacco (pan masala) in Assam. Numerous studies have reported that the constituent of BN/BQ exposure to be significantly associated with susceptibility to oral and oropharyngeal cancers [31]. There are various types of elements present in BN that includes crude fiber, carbohydrates, fats, polyphenols, alkaloids, tannins, proteins and water. Trace amounts of fluorine, sapogenins (glycosidic derivatives of steroids) [31], slaked lime in betel quid causes inflammation in the sub-mucosal area. Calcium hydroxide content of lime in the occurrence of the areca nut is primarily responsible for the development of reactive oxygen species that might cause oxidative damage in the DNA of buccal mucosa cells in betel quid chewers [32]. The nitrosation of arecoline might produce a variety of betel quid-specific nitrosamines which can interact with DNA, proteins or other targets forming adduct to exert its carcinogenic activity.

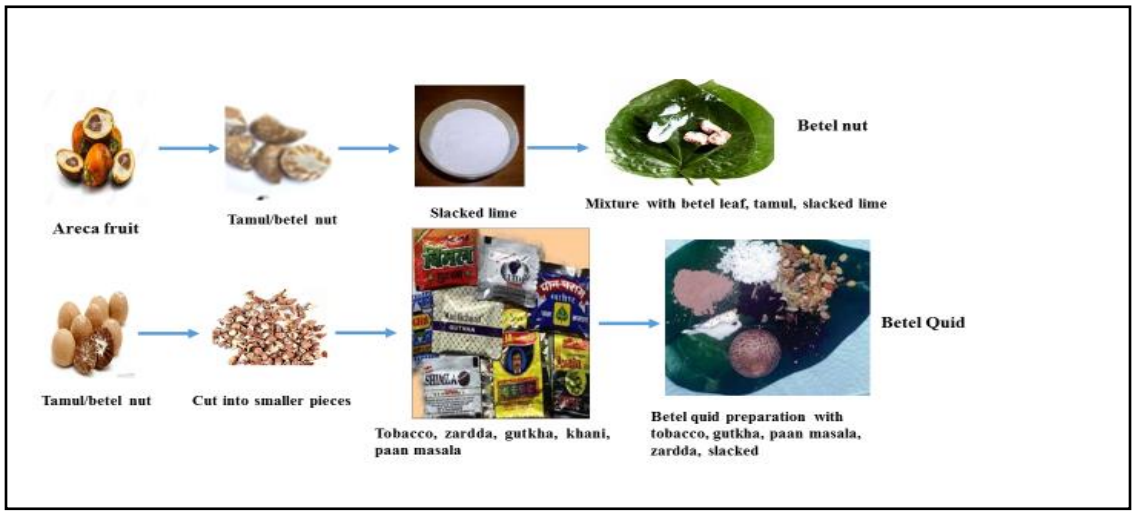

Figure 5: Betel nut/ tamul pan and betel quid (BQ) preparations/product widely available in India market [31].

Table 5: Major carcinogenic and genotoxic agents in pan masala and gutkha [33].

Products Ingredients	**Genotoxic**	**agents/carcinogens**
Gutkha	Tobacco	NNN, NNK
	Areca nut	arecoline, MNPN
	Areca nut	lime ROS
	Catechu	lime ROS
Pan masala	Areca nut	Arecoline, MNPN
	Areca nut	lime ROS
	Catechu	lime ROS

Abbreviations: NNN, N'-nitrosonornicotine; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1- butanone; MNPN, 3-(methylnitrosamino) propionitrile; ROS, reactive oxygen species, O±´, H_2O_2, OH.

The carcinogenic TSNAs N'-nitrosonornicotine (NNN), 4-(N-methyl-N-nitrosamino)-1- (3-pyridyl)-1-butanone (NNK), 3-(methyl-N-nitrosamino), propionitrile (MNPN) and N-nitrosoanabasine (NAB), as well as the volatile nitrosamines N nitrosodimethylamine and N-nitrosodiethylamine, have been detected in the saliva of chewers of BQ with tobacco. It has been suggested that TSNAs undergoing metabolic activation may involve the cytochrome p450 system and other enzyme. NNK, a major carcinogenic agent to develop various types of cancer [33].

Reactive oxygen species (ROS), occupied in multistage carcinogenesis, are generated in substantial amounts in the oral cavity during chewing. The cellular metabolism of BN or BQ components may also generate ROS, such as superoxide anion radicals (O^{2-}) and hydrogen peroxide (H_2O_2) at pH greater than 9.5 [31]. Saliva was found to inhibit both O^{2-} and H_2O_2 formation from BQ ingredients. The addition of BN/BQ with slaked lime constituting ROS formed in the alkaline chewing mixture within the saliva cause cytotoxicity damage in hamster check pouch and may contribute to the cytogenetic damage found in the oral cavity of BN/BQ exposure [31, 34].

Endogenous Nitration and N-Nitrosamines Carcinogenicity

Endogenous nitrosation occurs during BQ chewing, exposing BQ chewers to four N-nitrosamines derived from arecoline. The arecoline-derived N-nitrosamines (AN) includes 3-Methylnitrosaminopropionitrile (MNPN), 3-

Methylnitrosaminopropionaldehyde (MNPA) and N-nitrosoguvacine (NGC) are undetectable in the areca nut (AN) before chewing and are formed by the endogenous nitrosation of arecoline. Exposure to nitrosamine carcinogens formed by endogenous nitrosation is higher among BQ chewers who swallow the BQ juice [35]. Numerous case-control studies have also reported that swallowing the BQ juice is associated with a significant increase in the risk of oral cancer [36]. Urinalysis of Syrian hamsters fed with AN and a nitrite sources detected N-Nitrosoguvacoline (NGL) and its metabolite, N-nitrosonipecotic acidrepresenting that exposure to nitrosamine carcinogens formed by endogenous nitrosation is likely higher among BQ chewers swallowed the BQ juice [35].

2.4.2 Tobacco

Tobacco is one of the world's most preventable cause of cancer. Survey from WHO calculated that 5.6 trillion cigarettes smoked per year at the predicted rate will lead to approximate 10 million mortalities by 2030 [37].

According to epidemiology survey evidence in developing countries, smokeless tobacco is used in various forms like khaini, mava, zarda, ghutka, etc. [38]. Mostly, people chew or suck (dip) tobacco in their mouth and spit out the tobacco juices that build up; even "spitless" tobacco has been developed. Several reports give evidence of the use of tobacco in many regions and countries, such as North Europe, North America, Africa, Asian countries and India [39]. About 28 types of chemicals were found in tobacco that can cause cancer. Most harmful compound in tobacco such as nitosamines, aldehyde,4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), N′-nitrosonornicotine (NNN), and 4-(methylnitrosamino)-1-(3- pyridyl)-1-butanol (NNAL), polycyclic aromatic hydrocarbons (PAHs), ethylene oxide, 4-aminobiphenyl, benzo(alpha)pyrene (BaP), upon potential exposure can induce cellular and tissue damage through direct cytotoxic effects, and their promotion of genotoxic events or the generation of reactive oxygen intermediates and DNA adducts [38-41].

2.4.3 Smoking

Tobacco smoking is associated with heart diseases, and cigarette smoking habits is the major cause of lung cancer death in worldwide [42]. The WHO reported that daily on

an approximate, 1 billion men and 250 million women in world are exposed to tobacco smoking. Therefore, 90% of lung cancer cases can be attributed to cigarette smoking [42, 43]. It is the leading cause of cancer related death worldwide with approximately 1.2 million deaths annually. It accounts for 30% of all cancer cases in developed nations [43]. According to International Agency for Research on Cancer (IARC) working group monograph in 1986, it was found that active tobacco smoking has carcinogenic effects on humans, and concluded that tobacco smoking caused cancer of lungs, bladder, upper aero-digestive tract, oral cavity, pharynx, larynx, and esophagus; and pancreas [44]. Even smoking cigarettes, pipes, cigars or bidi (which contain of a small amount of tobacco wrapped in the leaf of another plant, and are commonly used in South Asia) can also cause cancer of the paranasal, nasal cavity, sinuses, nasopharynx, liver, stomach, kidney, cervix uteri and adenocarcinoma of the esophagus and myeloid leukemia [43].

Tobacco smoke contains more than 60 compounds which are classified as carcinogen and at least 16 unburned compounds, including specific nitrosamines (such as 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and N′-nitrosonornicotine (NNN), aromatic amines (such as 4-aminobiphenyl) and polycyclic aromatic hydrocarbons (such as benzo[a]pyrene) that appear to have contributory roles in induction of cancer [43].

Smoking increases the activity of certain enzyme in the lung such as aryl hydrocarbon hydroxylase (AHH) and ethoxycoumarin-O-deethylase (ECDE), and decreases other enzyme activities in the lung that mainly plays role in cancer incidence [42]. Based on carcinogenicity studies in laboratory animals, biochemical evidence from human tissues and fluids, and epidemiological data, NNAL, 4-(methylnitrosamino)-1-(3-pyridyl)-1- butanol; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNN, N′-nitrosonornicotine; PAH (polycyclic aromatic hydrocarbons) were found to have mutagenic effect, which may play a role in tumorogenesis at this site [46].

Table 6: Tobacco associated Carcinogens and genotoxic in human organ in which tobacco or tobacco smoke cause cancer [43].

Type of Cancers	**Cancer type Likely carcinogen involvement**	**Genotoxicity**
Lung	PAH, NNK (major) 1,3-butadiene, isoprene, ethylene oxide, ethyl carbamate, aldehydes, benzene, metals	Chromosomal and gene mutations
Laryngeal	PAH	TP53 and P16 mutations, LOH
Nasal	NNK, NNN, other nitrosamines, aldehydes	Micronuclei, chromosomal instability, DNA strand breaks
Oral cavity (smokers) (smokeless- tobacco users)	PAH, NNK, NNN NNK, NNN	Micronuclei, chromosomal instability, DNA strand breaks
Oesophageal	NNN, other nitrosamines	TP53 mutations
Liver	NNK, other nitrosamines, furan	No data
Pancreatic	NNK, NNAL	KRAS and other mutations
Cervical	PAH, NNK	Mutagenic mucous, epithelial micronuclei
Bladder	4-Aminobiphenyl, other aromatic amines	Mutagenic urine, cytogenetic changes, DNA strand breaks
Leukemia	Benzene	No data

Based on carcinogenicity, NNAL, 4-(methylnitrosamino)-1-(3-pyridyl)-1- butanol; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNN, PAH, polycyclic aromatic hydrocarbons, N′-nitrosonornicotine;

2.4.4 Alcohol

The term ‘alcohol’ is the common name for ethanol or ethyl alcohol, a chemical compound found in wine, beer and liquor, as well as in some medicines, mouthwashes etc. Alcohol is produced from the fermentation of sugars and starches by yeast. The IARC of the World Health Organization (WHO) has classified alcohol as a Group 1 carcinogen [21]. Based on the data from 2009, an estimated 3.5% of all cancer deaths in United State occur due to alcohol consumption [46].

Alcohol consumption is considered as one of the strong risk factor associated between several types of cancer such as head and neck (particular oral cavity lip, pharynx, and larynx) [47], esophageal [47], liver, breast, pancreas, ovary, prostate, stomach, uterus and colorectal cancer etc., respectively [47, 48].

Alcohol, a major component of alcoholic beverages, is oxidized to acetaldehyde by alcohol dehydrogenases (ADH). Acetaldehyde is metabolized to acetate by aldehyde dehydrogenases (ALDH) [49, 50]. ALDH2 enzyme is crucial role involved in the alcohol metabolizing pathway. Previous studies have demonstrated that acetaldehyde has carcinogenic and mutagenic role in human. Acetaldehyde can damage both DNA (the genetic material that makes up genes) and proteins [51].

2.4.5 Human Papilloma Virus (HPV)

Human papilloma virus (HPV) is identified as an etiological factor of a subset of head HNC [52]. The HPV are small double-stranded DNA viruses that include a heterogeneous family involving of more than 130 different HPV types [53]. High-risk HPV (hr-HPV) types are significantly associated to viral neoplasm, incidence accounting for nearly 600,000 cases (5%) of cancers worldwide annually [53].

However, hr-HPV positive cancer cases accounts for approximately30-65 % of all HNC and 50-80 % of hr- HPV cancer cases arising in the oropharynx. HPV 16 and 18 are the two most prevalence HPV types in HNC cases [52]. It is frequently observed in cervical cancer (50%) and more than 90% in oropharynx cancer cases [53]. The epidemiology research have suggested numerous environmental risk factors such as tobacco and alcohol exposure and sexually transmitted infections are responsible for majority of HPV related HNC tumor [54, 55]. Many authors have reported that hr-HPV infection was associated with over expression of $p16^{INK4A}$ in tumor tissue [53, 55]. hr-HPV infection has been established to play an important role in the molecular pathways through its two important viral oncoproteins, E6 and E7. These are always found to be expressed in HPV associated cancer. The E6 protein activation lead to degradation of p53 via E3 ubiquitin E6AP [55] and E7 protein plays central role in both viral life cycle and carcinogenic transformation and inactivates the pRB protein

(retinoblastoma tumor suppressor) which controls the G1-S phase transition of the cell cycle. The E7 proteins also interrupt the close association between cellular differentiation and proliferation in normal epithelium with tumor initiation and induction of genomic instability [56]. The disruption of the pRb pathway is molecular marker with the best prognosis by using immunohistochemistry (IHC) technique in hr-HPV positive in tumor cases [53].

2.5 Genetic Polymorphism and Cancer

Recently, work on individual variation and its relevance as a predisposition factor had been gaining importance. Allelic variations in individual or in population also can have profound effect on predisposition of particular disease. Variations between individuals to cancer risk are generally presumed to be associated with variant alleles of different genes in a significant proportion of particular population. Genetic polymorphism in promoters enhances or down regulates the expression of genes, and exonic polymorphism determines the splicing patterns, iso-form expression, or extending the life span of transcripts.

2.5.1 Polymorphism in Xenobiotic metabolism enzymes

Most carcinogenic compound requires metabolic activation by phase I enzyme (CYP-450, EPHX1) and detoxification by phase II enzyme (GSTs, NAT etc.) through conjugation [57]. The phase I and Phase II enzymes are various groups of proteins that are responsible for metabolizing enormous number of compounds such as steroid, fatty acid, including xenobiotics and mutagenic compounds. However, the phase I and Phase II enzymes frequently exhibit genetic polymorphism, resulting in changing of the enzyme activity of the xenobiotic genes that have profound effect on the risk for various cancers [57, 58, 59].

2.5.2 Cytochrome P450 (CYP)

The most important Phase I drug metabolism enzymes belongs to the cytochrome P450 (CYP) super family [58], that catalyzes the oxidation reaction of large amount of the carcinogenic compound including drug, toxic, etc. The CYP450 systems comprises

57 genes and among them CYP1, CYP2 and CYP3 are the major player of oxidative metabolism pathway [59].

CYP1A1: Human CYP1A1 participates in the reaction of Phase I group [59]. These are the key enzymes which causes biotransformation of polycyclic aromatic hydrocarbons (PAHs), aromatic amines, estrogens and food mutagens formed during the pyrolysis of creatine, amino acids [58] The CYP1A1 gene, located at 15q22-q24, spanning 5,810 bp with seven exons and six introns [58]. The variant of the genetic polymorphism of these genes lead to the variation in enzyme activity that are associated with numerous cancer such as breast, lung, oral, colon, ovarian and acute lymphoblastic leukemia [58, 60, 61, 62]. More than 11 allele of CYP1A1 have been recognized of which CYP1A1*2A, *2C, *3, *4, *5, *6, *7, *8, *9, *11 show amino acid changes [63]. The most common genes existence CYP1A1*2A or m1 (3798T>C), also known as Msp1, CYP1A1*2C or m2 (2455A>G), CYP1A1*3 or m3 (3204T>C) andCYP1A1*4 or m4 (2452C>A) polymorphisms for CYP1A1 [58].

In Indian population frequency of CYP1A1*2A and CYP1A1*2C of the mutant allele were shown significantly (<0.05) associated with cancer comparison with other population [58]. The CYP1A1*3 was absent in Indian population, although CYP1A1*2B (9.5%) was determined only in NE population [58, 64]. The risk for HNC in subjects with variant CYP1A1 Ile-Val substitution at codon 462 alleles shows that, [65], the risk for Ile/Val and/or Val/Val genotypes was significantly higher than that for subjects with the Ile/Ile genotype, suggesting that the Val allele may be associated with increased risk for HNC [66].

CYP2E1: The CYP2E1 enzymes are involved in bioactivation of numerous pro-carcinogens and pro-toxin such as (N nitrosodimethylamine, styrene, benzene and N-alkylformamides) and the volatile anaesthetics (sevoflurane, enflurane, methoxyflurane, halothane and isoflurane drug) [67]. The enzyme is inducible by starvation and alcohol, and thus, these reasons influence affect toxicity or carcinogenesis due to alterations in CYP2E1 activity [68]. The CYP2EI gene is located on human chromosome 10q24.3-qter [68]. There are 12 different SNPs that have been identified [58, 69]. The genetic polymorphism of CYP2E1 have been associated to

numerous chemical induced carcinogenesis and to alcoholic liver disease, in particular liver cirrhosis [58].

Previous Case-Control studies have reported two types of polymorphic gene of CYP2E1 exist that possibly increase risk for cancer. One of these is detectable with RSa I enzyme by PCR-RFLP method which, alters the regulatory region of the gene, affecting transcription levels and presumably decreasing metabolism of nitroso compounds [68]. The second can be detected by Dra I enzyme in intron 2, which are associated with a decreased incidence of lung cancer in Japanese and Swedish populations [68, 70, 71]. The polymorphic CYP2E1 *1B allele mutation in intron 7 and the 5'flanking region CYP2E1*5B (C1/C2, Rsa I) and intron 6 CYP2E1*6 (C/D, Dra I) are the common variants associated with altered gene function and expression. Their prevalence has associated with altered gene function and expression [58]. The CYP2E1*1B frequency are equally distributed in North India, South India and Caucasians population, while it was significantly higher in Asians, 18.1 per cent ($P < 0.05$) and relatively highest in Africans, 65.9 per cent ($P < 0.001$). Although, the CYP2E1*5B gene frequency in North Indian population was different from other ethnic populations whereas *6 was similar to Asians but different from Africans and Caucasians. [58, 72, 73, 74]. Many studies have suggested that the c1/c2 genotype of CYP2E1 may increase risk for HNC compared with the c1/c1 genotype [75, 76, 77].

2.5.3 Glutathione S-transferases (GST)

The Glutathione S-transferases (GST) is a large group of dimeric isoenzyme GSTM1, GSTT1 and GSTP1 that play critical role in the detoxification of oxidative stress, free radicals, tobacco smoke product, and carcinogens such as polycyclic aromatic hydrocarbons (PAHs), benzo pyrene and other [78, 79]. Polymorphisms exist within the GSTM1, GSTT1 and GSTP1 genes that are located in chromosome as GSTM1 (μ) chr1p13.3, GSTT1 (θ) chr22q11.2 and GSTP1 (π) chr11q13.2 [58].

Previous report suggested that both the GSTM1 and the GSTT1 null variants result in the lack of enzyme production [80]. The polymorphic from of GSTM1 and GSTT1 genes are deletion variants that produce either a functional enzyme (non-deletion alleles or heterozygous deletion) or complete absence of the enzyme (homozygous

deletion alleles) [79]. However, these GSTs enzyme individually (GSTM1 and GSTT1) or in combination (GSTM1 + GSTT1) of the null genotypes of GSTM1 and GSTT1 genes increases the risk of lung, gastric, colon, bladder cancers [58, 81], the role of polymorphic GSTP1 genes, 313A>G of *GSTP1* (Ile105Val) in exon 5 has been reported to result in reduce enzyme activity [58, 81].

The *GSTM1* null genotype of frequency distribution in Indian populations was significantly different from Africans, Asians and Caucasians (P <0.03) [58]. Similarly, the frequency of *GSTT1* null polymorphism in South India was in link with North India and different from North East India (P<0.01). Moreover, in Indian populations the prevalent frequency was less significant as compared to Africans and Asians (P <0.001), but showed similarity with Caucasians [58, 82, 83]. Further, The *GSTP1* gene of Ile to Val substitution at codon 105 (exon 5) has been identified to increase the risk of HNC [84]. In Africans population were observed significantly difference (P <0.0001) as compare to North India and North East India population [58].

2.5.4 Microsomal epoxide hydrolase (mEH) (EPHX1)

Human microsomal epoxide hydrolase (mEH) (EPHX1) is phase I enzyme [85]. It plays dual key role during both activation and detoxification of exogenous chemicals such as polycyclic aromatic hydrocarbons (PAHs), which are produced during the use of coal tar, coke, bitumen or during cigarette smoking and highly toxic and mutagenic [85, 86]. Previous study have shown that the role of EPHX1 in carcinogenesis depends on the exposure of different environmental substrates [87]. The EPHX1 gene, is located on chromosome 1q42.1 is expressed in nearly all human tissues and their length is 35.48 kb with 9 exon and 8 intron [88]. More than 110 SNPs have been recognized in the EPHX1 gene [85]. Alteration in two amino acid related genetic polymorphism have been identified and associated with alteration of enzyme activity that leads to inter individual variations in the susceptibility to chemical carcinogen induced cancer which, are exon 3 (T>C, Tyr113His) and exon 4 (A>G, His139Arg) of EPHX1 [89, 90]. The EPHX His113 allele in exon3 has variant shows a 40% decrease enzyme activity, whereas the EPHX Arg139 variant shows 25% increase enzyme activity [86].

Several previous studies have evaluated that EPHX1 polymorphisms (Tyr113His and His139Arg) has been associated with chemical carcinogen- induced cancer of lung, liver, colorectum, ovary and HNC [85, 91]. The EPHX Arg139Arg homozygous variant differs between US Caucasian, British, German, African, American and Asian populations. In Indian population the Tyr113His genotype of exon 3 was associated with a higher risk of esophagus cancer, whereas the His139Arg genotype of exon 4 exhibited a lower and higher risk of esophagus cancer in Indian population [92, 93].

2.5.5 Arylamine N-acetyltransferases (NAT)

Human Arylamine N-acetyltransferases (NAT) are phase II xenobiotic metabolizing enzyme that plays key role in both activation and detoxification of procarcinogen compounds containing tobacco-derived aromatic and heterocyclic amine carcinogens or N-acetylation of arylamines, arylhydroxylaminesand arylhydrazines in the drug metabolism through N- and O-acetylation pathways [86, 94]. The gene encoding two isoform, NAT1 and NAT2 genes located on chromosome 8p21.323.1 and expresses two highly polymorphic isoenzymes, NAT1 and NAT2 with distinct functional roles [94, 95]. The NAT1 enzyme is found in the intestine, placenta, liver, bladder and breast [95], and is responsible for the alteration of the arylhydroxamic acids to the mutagenic acetoxy esters and O-acetylation of arylamine [96]. More than 15 NAT1 genotype have been identified [94]. Four major NAT1 genotype has been identified using polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) and allele-specific PCR method [97], are NAT1 ∗ 4, NAT1 ∗ 3, NAT1 ∗ 10, and NAT1 ∗11 [96]. NAT1 ∗4 (wild-type) allele, NAT1 ∗3 has a single C→A substitution (C1095A). NAT1 ∗10, which has two single-base mutations (T1088A and C1095A), and has been reported to have higher enzymatic activity [98]. A 9 bp deletion between nucleotides 1065 and 1090 in the untranslated region characterizes the NAT1*11 fast acetylator allele [94]. Numerous studies have reported that NAT1*10 allele associated with the risk of colon, pancreas, larynx, bladder and lung cancer [94, 98]. NAT2 enzyme is mostly expressed in the intestines and liver and is involved in the N-acetylation of arylamines by converting them to a non-reactive form [94]. In NAT2 *4 allele (wild type) and four major mutant allele are *NAT2* ∗5, *NAT2* ∗6, *NAT2* ∗7 and *NAT2* ∗14 [98]. The presence of two mutant alleles produce a slow acetylation activity and a

decreased half-life of the protein. The slow acetylator alleles are more frequent in the Caucasian population [94]. Previous studies report suggested the association between the NAT2 gene polymorphisms and risk of bladder, lung and breast cancer [94].

2.5.6 DNA Repair Genes

DNA repair system have a critical role for maintaining the genome from exposure to exogenous chemicals and physical agents (i.e., benzo[a]pyrene, dioxin, polychlorinated biphenyls, cigarette smoke, ultraviolet light, asbestos, radon), as well as endogenous mutagens [99]. If DNA damage is recognized by cell machinery, several responses may occur to prevent replication in the presence of genetic errors [100]. However, reduced DNA repair capacity results in apoptosis or can lead to unregulated cell growth and risk of cancer [100]. Genetic polymorphisms of DNA repair genes have been identified that lead to numerous types of cancers [86]. More than a hundred proteins involved in DNA repair pathway have been found in human cells. DNA repair genes are involved in four major DNA repair pathways, including base excision repair (BER), nucleotide excision repair (NER), double strand break repair (DSBR) and mismatch repair (MMR) [101].

Base excision repair (BER): BER pathway repairs small genomic lesions such as oxidized and many of the alkylated bases or reduced bases, fragmented or non-bulky adducts, or those produced by methylating agents [102]. The single damaged base is removed by base-specific DNA glycosylases; e.g., the oxidized base 8-oxoguanine is excised by 8-oxoguanine DNA glycosylase [100], which are often promiscuous as far as their substrate specificity is concerned. The BER pathway can proceed through two different sub pathways: long-patch and short-patch BER. These pathways are differentiated by the enzymes involved and the number of nucleotides removed. Long patch BER inserts 2–13 nucleotides by concordant action of polymerase δ, proliferating cell nuclear antigen, flap endonuclease 1, and ligase I [102]. The short patch BER replaces a single nucleotide by polymerase β and the newly synthesized DNA sealed by DNA ligase III/X-ray cross complementing group (XRCC1) heterodimer [103]. BER is responsible for repairing single-strand breaks. The X-ray repair cross complementing group 1 (XRCC1) gene is involved in the BER pathway [104]. Numerous study have

been reported that XRCC1 gene including Arg194Trp, Arg280His and Arg399Gln single-nucleotide polymorphisms show decrease in the kinetics of repair mechanism, and influences susceptibility to cancer [105, 106]. Individuals with the XRCC1 399Arg/Gln variant show increased sister chromatid exchange after treatment with a tobacco-specific carcinogen, NNK. However, previous reports showed that of XRCC1associated increased with HNC risk were inconsistent [86, 106].

Nucleotide excision repair (NER): The NER pathway is most versatile in DNA repair system that operates primarily in a wide range of DNA lesion including that caused by environmental mutagens, such as polycyclic aromatic, hydrocarbons and UV light [107]. In NER there are two sub pathways, one is transcription coupled DNA repair (TCR), which preferentially eradicates DNA damage that blocks ongoing transcription in the transcribed DNA strand of active genes. The second is global genome repair (GGR), which removes lesions throughout the genome, including those from the non-transcribed strand in the active gene [103, 108]. Xeroderma pigmentosum complementation group C (XPC) and excision repair cross-complementation group 6 are essential in the NER damage recognition step with different target specificity. However, in XPC Lys939Gln polymorphism might contribute to the development of bladder cancer risk [107]. Meta-analysis conducted by *Liu et al.* suggested that XPG Asp1104His polymorphism was not associated with breast cancer risk [109]. Several polymorphisms has been identified in the *XPD* gene, of which Lys751Gln is one of the maximum reported polymorphisms. There are meta-analyses indicating XPD Lys751Gln polymorphism might contribute to the risk of bladder cancer [107, 109]. Several study reported that the association between XPD Lys751Gln polymorphism and different types of cancer risk in diverse populations, lung cancer, breast cancer, gastric cancer, colorectal cancer [110]. Similarly the XPD genotype increased HNC risk in CC homozygotes compared with AA homozygotes [106].

Double-strand break repair (DSBR): DSBR is produced by replication error and by the most exogenous agents such as ionizing radiation and mutagenic compounds in DNA damage. In case of a single unrepaired DSBR is often sufficient to cause cell death [111]. In addition, incorrect repair can lead to deletions or chromosomal

aberrations; this is associated with the development of cancer or other genomic uncertainty syndromes [111, 112]. Therefore, the repair of DSBs is not error in DNA repair pathway is critical for cell survival and maintenance of genome integrity [111, 112]. The two main mechanisms of double-strand-break repair exist in mammalian cells repair DSBs are homologous recombination (HR) and non-homologous end-joining (NHEJ). In homologous recombination directed repair is mainly an error-free mechanism as it utilizes the genetic information contained in the undamaged sister chromatid as a template [111, 113]. This pathway is thought to contain more than 16 molecules including products of the breast cancer genes such as *BRCA1* and *BRCA2* and *XRCC3* [114]. In contrast, non-homologous end-joining repair pathway error-prone involves direct ligation of the two double-strand-break ends and also involves numerous molecules, including LIG4. The NHEJ is predominate pathway in mammalian cells, which control all phases of cell cycle. In case of HR is restricted to the cell cycle of late –Sand G2 phases [111].

Mismatch repair (MMR): The MMR system plays a crucial role in post-replication repair of mis-incorporated bases that have escaped the proofreading activity of replication polymerases [101, 111]. It corrects replication error, mismatched bases and also corrects insertion/deletion loops (IDLs) that caused by DNA polymerase errors. MLH1, MSH2, PMS2, and MSH6 genes are involved with MMR pathway [111]. The direct reversal of DNA damage may activate O6 -methyguanine methyltransferase (MGMT), which removes a methyl group from the O6 -position in guanine and transfers it to its own cysteine residue at codon 145 in the protein [115]. While MGMT showed one independent repair mechanism, alkylating agents have taken advantage of other multi-enzymatic pathways to induce cell death. Several study suggested that the therapeutic action of alkylating agents would be more effective in an MGMT-deficient environment.

The DNA repair phenotype is genetically determined and consequently differs in general population for HNC [115-117]. However, previous reviewer reported that genetic polymorphism of MGMT gene have associated with increased risk for lung, breast, head and neck, colorectal and other types of cancers [115 -117]. The most

common genotype of MGMT gene are Ile143Val and Lys178Arg and 20% found in variation of disequilibrium [117, 118]. Other variations effecting MGMT gene includes Trp65Cys, Leu84Phe, Gly160Arg, Gly135Thr, Gly290Ala, Cys485Ala, Cys575Ala, Gly666Ala, Cys777Ala, Gly795Cys, Ala1034Gly and Cys1099Thr [115, 119]. Furthermore, pooled analysis of Leu84Phe SNP indicated that this single variant was not associated with risk of HNC [120].

2.5.7 Alcohol dehydrogenase

Alcohol consumption has been identified to cause a large number of health losses, chronic diseases and death worldwide [121]. A small portion of the alcohol is diffused into the bloodstream directly from the stomach wall, but maximum passes through the pyloric junction into the small intestine, where it is very rapidly absorbed [122]. Previous epidemiology studies reported that chronic alcohol consumption is a crucial risk factor for the increased risk to development of various types of cancer such as liver (hepatocellular carcinoma), colon or rectum, upper aero digestive tract (oral cavity, pharynx, larynx, pharynx), esophagus and breast [123]. The main role of ethanol metabolism was observed in tumor initiation is implicated by the association between various form of cancer and polymorphism in genes involved in the oxidation of ethanol [121].

However, people who consume of alcohol ≥ 50 grams per day have two to three times increased risk of cancer compared to the non -alcohol consumption [124], and synergistic effect is seen among alcohol and tobacco consumers [125]. More than 100 epidemiological studies have constantly reported that doses of alcohol increased the risk for breast cancer [122].

The mechanisms of ethanol- induced carcinogenesis are connected to the metabolism of ethanol. The two enzymes are involved in metabolism of alcohol, alcohol dehydrogenase ADH1B (ADH) and aldehyde dehydrogenase-2 (ALDH)-2 [122]. Acetaldehyde is produced mainly from ethanol via oxidation by alcohol dehydrogenase (ADH) and is subsequently detoxified into acetate by aldehyde dehydrogenase (ALDH) -2, respectively, in the liver [106]. The high level acetaldehyde is produce

from alcohol exposure which, are responsible for increased risk of cancer such as HNC [106].

ADH holoenzyme may exist as a homodimer or a heterodimer combination of ADH1-α, ADH2- β and ADH3-γ subunits each of which is encoded by a distinct locus [125, 126]. A polymorphic ADH2 at codon 47 in exon3, resulting in an amino acid transition arginine (*Arg*) to histidine (*His*), ADH2*2 allele (*Arg47His*) is mutant which is highly prevalent in more than 90% of East Asians, but in fewer than 20% of either Caucasians or Africans [125]. In previous study demonstrated that about 40 times maximum velocity has been identified for the fast His/His (ADH2*2 genotype of ADH2 gene as compared to the less active Arg/Arg (ADH2*1) [122]. The less active ADH2*1/2*1 genotype among alcoholic is more prevalent (31%) as compare to the normal control (7%). Similar gene related tendency result has been reported in Japanese and Chinese patients with alcoholic liver disease, compared with normal controls [125, 127]. The ADH2*2 (His/His) allele shows a clear east-to-west decline where specifically it is dominant in East/West Asia, moderate in Southeast Asia. The ADH2*2 allele has a protective effect against heavy drinking and alcoholism in white population [125].

The ADH3 gene brings two alleles, coded of enzyme subunit, ADH3*1 -γ and ADH3*2-γ, that have different *in vitro* kinetic properties [128]. The ADH3*1 isoenzyme encoded allele metabolise ethanol into acetaldehyde is 2-5 times faster as compare to the encoded by ADH3*2 allele. The previous studies reported that the frequency distribution of ADH3*1 allele is 75-90% in Africa and 85-1000% in Asian population. Other study showed that the ADH3*1/2 or ADH3*2/*2 possess increased risk (13-times and 24-times, respectively) of esophagus cancer with moderate to heavy alcohol consumption in Asian population [128, 129].

ALDH2 is a polymorphic gene, and an individual's allele at this locus determines blood acetaldehyde concentration after alcohol consumption [106]. Most of the acetaldehyde formed during alcohol metabolism in liver is quickly eliminated by the low-Km mitochondrial ALDH-2 (ALDH2), [106, 130]. A single point mutation exist in ALDH2 polymorphic are ALDH2*2 allele Glu487Lys) encodes a catalytically inactive subunit. Previous study reported that the ALDH2*2 allele is rare in Western

populations but prevalent in East Asian populations including Chinese, Korean, Thai, and Japanese populations. In context, to prevalent of Asians population (frequency of up to 40%), it does not exceed 5% in European and African populations [116, 128]. Homozygous of *ALDH2**2/*2 allele have serum acetaldehyde levels that are 13 fold higher and heterozygotes have levels 4 times higher compare to the ALDH2*1*1 homozygotes [131]. The relative risk possess byALDH2 polymorphism *i.e.* ALDH2*1/*2 for HNC were 67%, in Japanese populations as compared with ALDH2 *1/*1 homozygotes [106]. In European population who consumed moderate or heavy alcohol consumption a highly significance interaction was reported for cancer of upper aero digestive tract [132, 133].

Thus the above studies specifies that genetic variant of many types of genes induces susceptibility to cancer. Therefore there is urgent need to identify susceptible genotype among mass population who are/has been exposed to exogenous carcinogens in the form of betel nut, tobacco, smoking and alcohol consumption. Though the prevalence of HNC is highest malignancies in NE India, few information are available on the association between genetic polymorphism and gene- gene interaction of the risk of HNC on NE India.

Therefore, the proposed work tries to identify the polymorphic status of selected genes and expression patterns that could act as causative factor for disease progression. Every population has defined genetic makeup which makes them susceptible to certain disease pathologies. To identify the risk genotypes in North Eastern region as well as the role of dietary habits in this population would give valid information to define the severity of disease and also helps in defining the effective therapeutic regimens for the management of disease.

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