# 3 Etiology of Head and Neck Cancer

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## Background Molecular Biology

The fundamental concept underlying cancer development is 'mutation' or damage to the genes of a cell. The accumulation of genetic alterations enables the clonal expansion of transformed cells, which may or may not lead to a malignant phenotype. Carcinogenesis can, therefore, be seen to be a multi-step process involving both the genotype and phenotype of a cell. When this process occurs at a phenotypic level, it is known as 'tumor progression' and describes events that are normally associated with malignancy, such as invasion or metastasis.

The molecular model proposed by Fearon and Vogelstein for colorectal tumorigenesis can be applied to most solid tumors including those of the head and neck.[1] They suggest that firstly, a carcinoma occurs because of the inactivation of tumor suppressor genes or the activation of proto-oncogenes. Clonal expansion of these transformed cells occurs before a defined set of genetic events leads to the development of the malignant phenotype. However, the order of progression varies between individual tumors, so that it is the net accumulation of genetic alterations that determines the malignant phenotype.

Further work has allowed a model for the progression of head and neck squamous cell carcinoma (HNSCC) to be proposed (Fig. 3.1).[2] Loss of heterozygosity (LOH) or allelic loss at the genetic locus 9p21 appears to be the commonest genetic change, resulting in the inactivation of the tumor suppressor gene p16 which encodes a cyclin-dependent kinase inhibitor (prevents cell proliferation by arresting the cell cycle in G1 stage). This was identified in 20% of benign squamous hyperplastic lesions suggesting that it occurs early in the progression of head and neck tumors. Another frequent mutation is LOH of the p53 gene located at 17p13. This is one of the commonest mutations found in all forms of human cancer and results in a progression from preinvasive to invasive lesions and increases the likelihood of further genetic progression.[3] Further detailed analysis has identified other tumor suppressor genes and proto-oncogenes which can occur early, intermediate or late in the development of head and neck carcinogenesis (Table 3.1).

This model demonstrates that benign squamous hyperplastic lesions can contain cell populations with clonal genetic changes. This supports the concept of 'field cancerization' which was first suggested by Slaughter et al in 1953. In this study, normal epithelium from upper aero-digestive tract carcinomas was found to have altered histology suggesting that the entire region's mucosa had undergone a change related to carcinogen exposure.[4] This increases the tissue's risk of developing several independent premalignant and malignant foci and helps to explain why multiple primary and second primary tumors occur in HNSCC patients. A different explanation is that multiple tumors share a clonal origin and migrate to different sites where they subsequently acquire distinct genetic changes. Whatever the cause, second primaries are a major threat to the long-term survival of HNSCC patients following successful treatment of early-stage lesions.

A mathematical model estimating the number of genetic events required for the development of different types of cancer has suggested that 6–10 separate DNA damaging incidents contribute to the development of squamous cell carcinoma in the head and neck region.[5]

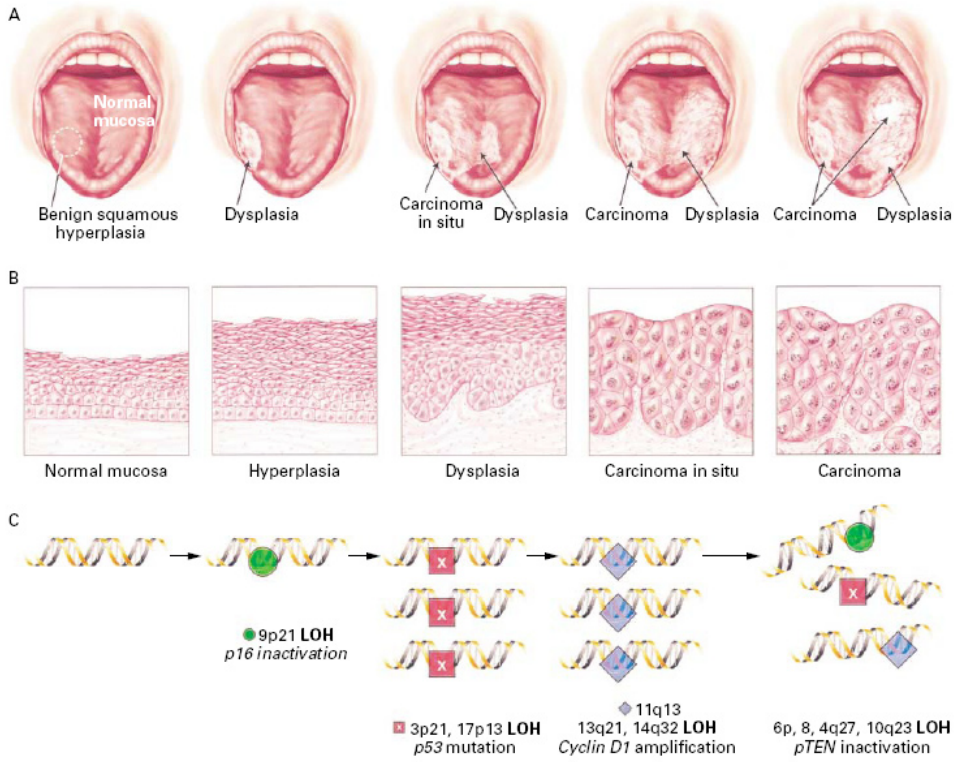


***Fig. 3.1*** *A model demonstrating the molecular progression of head and neck cancer with its clinical and pathological correlates (published with permission)* [2]

This is probably more than is required for development of other solid tumors and perhaps explains the long history of carcinogen exposure in HNSCC and why a latency period exists between exposure and disease.[6]

## Genetic Predisposition

Whilst it is generally accepted that environmental factors play a large part in head and neck cancer development, it is likely that some of these genetic mutations are inherited, because not all individuals who are exposed to carcinogens like tobacco, go on to develop cancer. Several large case-control studies have demonstrated an association with family history with adjusted relative risks of 3.5–3.8 for developing HNSCC if there was a first-degree family history of HNSCC, which increased to 7.9 in relatives of patients with multiple primaries.[8–9]

Attention has focused on inherited differences in DNA repair systems and metabolising enzymes which would clearly influence a subject's susceptibility to potential environmental carcinogens. One group of enzymes involved in the detoxification of tobacco-related

*Table 3.1 Oncogene alterations identified in Head and Neck Squamous cell carcinoma (published with permission)* [7]

| Gene | Frequency (%) | Function |
|---|---|---|
| **Tumor suppressor genes** | | |
| $p16^{INK4A}$ | 80 | Senescence, cell-cycle progression |
| p53 | 50 | Cell-cycle regulation, cell survival |
| pTEN | 10 | Signaling, migration |
| Rb | <10 | Cell-cycle regulation, apoptosis |
| **Proto-oncogenes** | | |
| Cyclin D1 | 30 | Cell-cycle regulation |
| p63 (p40/p51/AIS) | 30 | Unknown |
| Epidermal growth factor receptor | <10 | Cell proliferation, growth |

carcinogens are the glutathione S-transferases (GSTs) and indeed their activity has been found to be suppressed in HNSCC patients.[10] Several polymorphisms exist in the different GST subtypes and there is some evidence to suggest that some of these are associated with a higher risk of HNSCC cancer development.[11] However, a meta-analysis of GST polymorphisms has demonstrated inconsistent results.[12]

Another such detoxifying enzyme is UDP-glucuronosyl-tranferase 1A7 (UGT1A7). A low activity has been shown to be associated with an increased risk of developing orolaryngeal cancer both in light (odds ratio, OR 3.7) and heavy smokers (OR 6.1) and furthermore, was not associated with any increased risk in non-smokers.[13] One recent report has even demonstrated a germline p16 mutation (p16R87P) in a family with high incidence of HNSCC in which the p16 allele was non-functional. This direct link, they suggest, represents a new clinical entity of familial head and neck cancer.[14]

This is an expanding area of research and it is likely that clear genetic susceptibility for the development of head and neck carcinoma will be identified. It must be stressed though, in terms of HNSCC prevention, that nothing would have as great an impact as reducing worldwide tobacco consumption.

# Environmental Factors

The head and neck region or more precisely the upper aero-digestive tract is, by nature of its function, exposed to an immense variety of environmental agents. Those that are carcinogenic or potentially carcinogenic include chemicals, viruses or radiation.

## CHEMICAL CARCINOGENS

### TOBACCO

It has been estimated that tobacco is responsible for nearly one-third of all cancer deaths worldwide. The overall risk is related to total lifetime exposure which itself includes the amount of tobacco a person smokes each day, the intensity of smoking (the size and frequency of inhalation), the age at which smoking began, the number of years a person has smoked, and a smoker's second-hand smoke exposure. It exhibits a clear linear dose–response carcinogenic effect in which duration is more important than the intensity of exposure.

The link between tobacco smoking and head and neck cancer was first suggested in 1915 when Abbe et al reported that smoking and alcohol consumption were common amongst patients with oral cancer.[15] It was not until 1957 that this association was first investigated by a case-control study.[16] Tobacco was finally designated as being carcinogenic for the head and neck region by the International Agency for Research on Cancer (IARC) in 1986. The confirmed associations included cancer of the oral cavity, oropharynx, hypopharynx and larynx.[17] In 2002, the association was extended to the remaining sites of the upper aero-digestive tract, namely, nasal cavity, paranasal sinuses and nasopharynx.

Today, despite the overwhelming evidence against smoking, more than one thousand million people worldwide smoke tobacco. While prevalence has declined in developed countries, it is on the increase in many developing countries. This is largely due to very successful marketing campaigns by tobacco companies with little or no governmental policy on raising awareness about the hazards of smoking.

#### Mechanism of action

Tobacco smoke contains approximately 4000 different chemicals including about 60 that are known to be carcinogenic. Nicotine is the principal component responsible for addiction. It is absorbed very quickly into the bloodstream from smoke that is inhaled into the lungs, with a cigarette having a total nicotine content of approximately 8mg. The major carcinogens are found in the particulate or tar fraction, which consists of a cocktail of cancer initiators, promoters and co-carcinogens. The major carcinogens are the tobacco-specific N-nitrosamines (TSNA) which are formed by N-nitrosation of nicotine and other tobacco alkaloids. The most powerful of these are N-nitrosonornicotine (NNN), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL).[18] Following metabolism, these carcinogens cause DNA alkylation, which can induce mutations. It is these mutations in key target genes which are often the initial events in cancer development. Other carcinogens include polycyclic aromatic hydrocarbons and catechols which are important co-carcinogens.

Whilst the main mechanism of cancer development by tobacco is through the direct action of chemical carcinogens, other indirect effects also deserve consideration. The most notable are the effects of tobacco smoke on the local mucosal immune system. Salivary IgA is lowered in smokers—those who are healthy as well as those who have HNSCC; the effect being dose-related and reversible on cessation of smoking.[19] Langerhans cells, which are antigen-presenting cells found in the epithelium (therefore essential for immunosurveillance),

are also in reduced number in smokers.[20] Other effects include increased production of salivary acetaldehyde (which is highly carcinogenic in vivo) by encouraging the growth of Gram-positive bacteria and other microbes like *Candida albicans*.

### Cigarette smoking

A number of epidemiological studies have attempted to quantify the risk of HNSCC due to cigarette smoking. In one large cohort study, the relative risk of upper aero-digestive tract cancers (including esophagus) was 4.7 for the highest level of smoking.[21] Various other case-control studies have demonstrated odds ratios from 6.5–13.0 for all HNSCC sites associated with ever smoking.[22–24] The risk is generally less if filter cigarettes and blond rather than black tobacco are used and the risk decreases dramatically following quitting. The level of tar in a cigarette is important with clearly increased risk for higher tar cigarettes.[25]

When the different sites are considered individually, by far the strongest association is with laryngeal SCC: estimates of risk are as high as 60 for those smoking more than 30 cigarettes/day.[26] Inhalation increases the risk of endolaryngeal cancer further but not that of hypopharynx or the epilarynx.[22] Table 2 shows the average relative risks associated with tobacco smoking, for the different sites in the head and neck, in studies considered by the IARC.[27]

*Table 3.2 Head and Neck Cancer Sites Associated with Tobacco Smoking*

| Cancer site | Average relative risk |
|---|---|
| Oral cavity | 4–5 |
| Oro- and hypopharynx | 4–5 |
| Larynx | 10 |
| Nasal cavity, paranasal sinuses | 1.5–2.5 |
| Nasopharynx | 1.5–2.5 |

### Cigar/Pipe smoking

Although cigar and pipe smoke contains many of the same carcinogenic compounds as cigarette smoke, variation in daily use and amount of inhalation does mean that differences occur in the type of HNSCC associated with this type of tobacco consumption. For example, majority of cigars users do not inhale the smoke because it is generally more irritating than cigarette smoke. For this reason, the strongest association appears to be with development of oral cancer.

Cigars also vary tremendously in size and quantity of tobacco. The biggest can be seven inches in length, containing as much tobacco as a packet of cigarettes and take up to two hours to smoke. It can, therefore, be hard to assess the level of exposure. Furthermore, cigar and pipe use is often combined with cigarette smoking making it difficult to analyse the individual risk associated with each.

Studies do confirm, however, that cigar and/or pipe smoking is independently associated with HNSCC. One large cohort study found UADT cancers were twice as likely in the cigar smoking group compared to non-smokers (relative risk 2.02, 95% CI 1.01–4.06).[28] The most important factors appear to be the extent of inhalation and as with cigarettes, the overall duration of exposure.

### Other forms of smoking

People, particularly in developing countries, smoke tobacco in many different ways. The risks associated with the various techniques are broadly similar to conventional cigarettes or in some cases worse, because the smoke is usually unfiltered. Like cigars, oral cancer shows the strongest association.

A common alternative to cigarettes in India is, *bidi* smoking. These are thin, hand-rolled cigarettes consisting of tobacco, wrapped in a *tendu* or *temburni* leaf and are often flavoured. They have higher levels of nicotine, tar and carbon monoxide than traditional cigarettes[29] and show similar relative risks for all-cause mortality compared to cigarette smokers.[30] Kreteks (from Indonesia) are similar and typically contain cloves as well as tobacco.

Reverse smoking is a common practice in some parts of India, where the lighted end of a cigar or cigarette is held in the mouth. Chutta, a homemade cigar or cheroot found in Southeast India, is often smoked this way. Reverse smoking appears to be particularly associated with development of cancerous lesions of the palate.[31] Other methods include the *hooka* (*nargile* in Arabic) which is common in the Middle East, and clay pipe smoking.

### Involuntary smoking

Over the last 20 years, it has become clear that environmental tobacco smoke (ETS) exposure or 'passive smoking' is associated with development of lung cancer. Meta-analysis has shown a significant and consistent association between lung cancer risk in spouses of smokers and their exposure to second-hand tobacco smoke. The IARC have recently added environmental tobacco smoke to their Group 1 list of known human carcinogens.[32]

Although data from studies looking at HNSCC is less clear, it is likely that involuntary smoking is associated with HNSCC development as well, albeit to a lesser extent. A case-control study by Zhang et al demonstrated a clear dose–response relationship with an adjusted OR of 3.6 (95 % CI 1.1–11.5) for individuals with heavy ETS exposure in comparison to those with no exposure. Other studies looking at HNSCC in non-smokers have found significant association with exposure to ETS.[33]

This has led to several high-profile legal cases, such as the 62-year-old non-smoking bar attendant in Australia who successfully sued her employers because she developed HNSCC. Moreover, several countries have now banned smoking in public places (e.g., USA, Canada and Ireland).

### Smokeless tobacco

Smokeless tobacco is essentially any tobacco that is not ignited for its use and includes chewing tobacco and snuff. Chewing tobacco is available in loose leaf, plug or twist forms. The user usually places a wad of tobacco inside the cheek which causes juices and saliva to collect and which are often spat out; hence the other name 'spit' tobacco. Snuff is a finely ground tobacco packaged dry or moist which the user places between the cheek and gum or sniffs into the nose.

The use of smokeless tobacco is widespread throughout Central, South and Southeast Asia and parts of Africa and encompasses types such as *nass*, *naswar*, *khaini*, *mawa*, *mishri* and *gudakhu*.[34] It is often chewed with other constituents such as areca nut and betel leaf (see section on betel quid). In Arab countries, *alshammah* and *alqat* are the traditional forms of chewable tobacco. In the US, smokeless tobacco (usually as oral snuff) is used by 3.4% of the population—most commonly by young men aged 18–25 years (male to female ratio 10:1).[35] In Europe, its use is confined to immigrant populations, except in Scandinavia where the use of oral snuff (snus) is again quite common.

Smokeless tobacco contains 28 known carcinogens.[36] The most harmful are, like those found in burnt tobacco, the tobacco-specific N-nitrosamines (TSNA) which are formed during the curing and aging of tobacco. Others include *N*-nitrosamino acids, volatile

*N*-nitrosamines, benzo(a)pyrene, volatile aldehydes, acetaldehyde, crotonaldehyde, hydrazine, arsenic, nickel and cadmium. The amount of nicotine absorbed from smokeless tobacco is three to four times the amount delivered by a cigarette. Nicotine is absorbed more slowly from smokeless tobacco than from cigarettes, but more nicotine per dose is absorbed from smokeless tobacco than from cigarettes and the nicotine stays in the bloodstream for a longer time.

As one would expect, all forms of smokeless tobacco appear to be carcinogenic in humans, with lesions typically developing at the site of application, mainly the oral cavity and occasionally in the nasal cavity. However, quantifying the risk has proved difficult. A recent systematic review has suggested that the only studies consistently showing a significant risk of oral cancer associated with smokeless tobacco, are from India; whereas studies from the US and Scandinavia are less conclusive.[37]


***Fig. 3.2*** *Examples of smokeless tobacco products found in the US and Scandinavia*

## ALCOHOL

Alcohol is the second most important global risk factor for head and neck cancer. It is involved in at least 75% of HNSCC, particularly cancers of the oral cavity, oropharynx, larynx and hypopharynx.[38] Extensive epidemiological evidence, both from cohort studies[39–41] and case-control studies,[22;42–45] suggests that it potentiates the carcinogenic effect of tobacco. This interaction appears to be multiplicative rather than additive, particularly with high levels of exposure.[46] There is also convincing evidence to suggest that alcohol is an independent risk factor for HNSCC, again predominantly at the highest levels of consumption.[40;47]

Estimates of risk of head and neck cancer in alcohol drinkers show increase in an exponential dose-dependent manner, with hypopharyngeal SCC often having the strongest association. In a large case-control study, Bruguere et al found a relative risk (adjusted for tobacco) of 13.5 for oral carcinoma, 15.2 for cancers of the oropharynx and 28.6 for the hypopharynx, in individuals with an alcohol consumption of 100–160

g/day (12.5–20 units/day). In a similar study of laryngeal cancer, Talamini et al found an adjusted odds ratio of 5.9 in the highest drinking category (>96 g/day).[48] Higher ORs were found in the supraglottic subsite (OR 11.7) compared to the glottis (OR 4.9). This study also confirms the huge multiplicative risk of combined heavy alcohol and tobacco intake with an odds ratio of 177.2 (95% CI 65.0–483.3) for laryngeal cancer.

Whilst moderate intake of alcohol (<30 g/day) does not confer any increased risk of HNSCC in males, it appears that females have an increased risk even at levels of 10–20g alcohol/day.[49] This is of particular concern in countries like the UK, where alcohol consumption at these levels is on the increase in women.[50]

Studies attempting to correlate specific cancer risk with the type of alcohol consumed, have been inconclusive. There does, however, seem to be some protective effect conferred by moderate intake of wine compared to beer or spirits.[40]

## Mechanism of action

It does not appear that alcohol is a carcinogen on its own. Studies with lifelong alcohol-exposed rodents have not shown an excess of tumor development compared with control animals.[51] In contrast, when it is administered along with known chemical carcinogens, tumor development does increase. It has, therefore, been described as a co-carcinogen or tumor promoter.[52]

The strength of association between alcohol intake and malignancy at different subsites within the UADT varies considerably. Hypopharyngeal SCC has the strongest association and tumors of the glottis and subglottis, the weakest. In the oral cavity, tongue and floor-of-mouth cancers have a stronger association with alcohol than palate and palatoglossal fold lesions: the so-called 'alimentary groove hypothesis' suggested by Lederman et al in 1964. This suggests that alcohol exerts a local, as well as systemic, co-carcinogenic effect. Table 3.3 summarises these various mechanisms.

## Systemic effects

Following absorption, alcohol undergoes metabolism which results in various toxic products including acetaldehyde, hydroxyl and ethoxy radicals. Acetaldehyde in particular is highly mutagenic and carcinogenic in animal models, interfering with DNA synthesis and repair by binding directly to DNA and cellular proteins.[53] It is formed in the liver by alcohol dehydrogenase (ADH) and can also be produced in the gastrointestinal tract by bacteria. Further oxidation by aldehyde dehydrogenase (ALDH) converts it to acetate.

Genetic polymorphisms in the enzymes metabolizing alcohol are associated with increased risk of developing UADT cancer. In Japan and other East Asian countries, a high percentage of the population carries a mutation in the ALDH2 gene. Individuals who are homozygous for this mutation have no ALDH2 activity, which results in elevated acetaldehyde levels following alcohol consumption and causes unpleasant

side effects such as flushing.[54] While these individuals consume little alcohol and do not have any increased risk of cancer development, those who are heterozygous for ALDH2 have only 30%—50% of normal enzyme activity, and do show increased risk of oropharyngolaryngeal cancer (adjusted OR 18.5).[55,56] Polymorphism in the ADH gene may also be associated with increased risk for HNSCC, through similar modulation of acetaldehyde levels. Although an IARC review did not show an increased risk of HNSCC for the ADH1C*1/1 or ADH1C*1/2 genotypes,[57] other studies have reported associations with ADH1B and ADH2 polymorphisms.[56,57]

Induction of the cytochrome P-450 2E1 or CYP2E1-dependent enzyme system occurs in chronic alcohol ingestion, which in turn may lead to increased activation of environmental carcinogens (nitrosamines, vinyl chloride, polycyclic aromatic hydrocarbons) from their pro-carcinogenic form.[50] This mechanism is likely to be partly responsible for the synergistic effect seen with

*Table 3.3 Proposed mechanisms of alcohol co-carcinogenesis*

| Local effects | Systemic effects |
|---|---|
| Solvent for potential carcinogens | Alcohol metabolism—acetaldehyde |
| Mucosal injury—aids carcinogen uptake | Production of other free radicals |
| Acetaldehyde production by oral bacteria | Chronic alcoholics |
| Carcinogenic impurities | — CYP2E1 enzyme induction |
| Chronic alcoholics | — nutritional deficiencies |
| — poor salivary flow | —altered retinoid metabolism |
| — gastro oesophageal reflux | —reduced immune surveillance |

heavy tobacco and alcohol consumption in HNSCC development.

Chronic alcoholics often suffer from vitamin and trace element deficiency through poor diet or otherwise, but the exact role of this in HNSCC development is unclear. Folate deficiency is common in alcoholics either through low intake or through destruction by acetaldehyde. This, in turn, may disturb gene regulation through inhibition of transmethylation.[58] Certain elements of vitamin A metabolism can also be affected, including reduced absorption and increased hepatic metabolism of retinoic acid precursors. Retinoic acid appears to be a protective factor in HNSCC.[50]

Disturbances in the immune system of alcoholics was first suggested thirty years ago when reversible defects in cell mediated immunity were identified.[59] Again, this is likely to be a multifactorial disorder related to malnutrition, vitamin deficiency and alcohol itself. In particular, the effect of alcohol on natural killer (NK) cells may be important, since these cells are cytotoxic to tumor cells. Reduced NK cell numbers and decreased lytic activity have been demonstrated in patients with alcoholic cirrhosis.[60]

### *Local effects*

The two most cited local effects of alcohol are: it acts as a solvent for carcinogens and injures the mucosa directly, causing cellular hyper-regeneration (which increases the susceptibility of the mucosa towards the action of carcinogens). It may also assist in the uptake of carcinogens.[61] Local production of acetaldehyde by oral bacteria is likely to be a factor, particularly in alcoholics who often smoke and have poor oral hygiene.[62] Other states associated with chronic alcoholism, such as salivary gland atrophy and gastro-esophageal reflux disease, may also contribute. In the former, a reduction in salivary flow leads to increased contact and higher concentrations of local carcinogens.[63] The issue of reflux is considered later in this chapter. Finally, carcinogenic impurities often present in alcoholic beverages (e.g., acetaldehyde, nitrosamines) although largely eliminated in production in developed countries, may be an issue in countries where local brewing is common practice.

## Betel Quid

The chewing of betel quid is an important risk factor for oral cancer throughout the Indian subcontinent, Southeast Asia, Melanesia and southern and eastern Africa. It is also commonly used by immigrant populations in Europe and North America.[64–66] It has been estimated that there are 600 million betel quid chewers worldwide.[67] Betel quid with tobacco was first designated as a carcinogen by the IARC in 1985 [68] and more recently betel quid without tobacco has also been classified as a human carcinogen.[69]

The composition of the quid varies from country to country, but it generally consists of a mixture of areca nut (*Areca catechu* Linn.), betel leaf (*Piper betle* Linn.) and slaked lime (calcium hydroxide). This may or may not be chewed along with tobacco. The areca nut itself is an astringent masticatory used to sweeten the breath, harden the gums and aid digestion. It also has medicinal uses as a taenicide to expel tapeworms.

Typically, the slaked lime is mixed with water to form a paste, which is then spread onto the *P. betle* leaf. The endosperm of the areca nut is then added along with dried tobacco leaves or stems. In most parts of India, the dried form of the areca nut is used, whereas in Taiwan and parts of Assam in Northeast India, young green nuts are preferred. The addition of lime tempers the acidity of the areca nut when it is chewed[70] and also enhances the release of psychoactive chemicals such as alkaloids.[71] Other ingredients can include catechu (*Acacia catechu*) and spices such as cloves, sandalwood, nutmeg, mace and peppermint. Finally, in most of Asia, the leaf is wrapped around the constituents to form the quid.

Over the last two decades, commercial betel quid substitutes have been aggressively advertised and marketed, paticularly in India. These products are flavoured and sweetened mixtures of areca nut, catechu and slaked lime with tobacco (*gutkha*) or without tobacco (*pan masala*).[72] They are simple to use, easy to carry around and are often claimed to be safer than traditional betel quid. Perhaps most worrying is the increasing use of these products in school children, teenagers and women.

The earliest reports that betel quid chewing was associated with development of oral carcinoma were

***Fig. 3.3*** *Betel quid preparation by a woman in Bhutan (published with permission)*

based on the site distribution of the tumors in chewers (typically buccal mucosa) compared to non-chewers.[73] Since then, actually defining the carcinogenic risk of betel quid has been difficult because of the confounding effects of tobacco. There are several combinations of exposure to consider: men often chew betel quid and tobacco and smoke as well, women may just chew either betel quid and tobacco or betel quid on its own while others may chew betel quid alone and smoke. One study that has distinguished between these exposures, and the different subsites in the head and neck, has confirmed that chewing betel quid and tobacco is an independent risk factor for development of all HNSCC.[74,75] It has also recently been shown that chewing betel quid alone is independently associated with oral cancer, adjusted odds ratio estimates ranging from 9.9–17.1.[76,77]

*Mechanism of action*

Oral submucous fibrosis (OSF) is a pre-malignant condition prevalent throughout South and Southeast Asia. The high transformation rate to oral cancer (7%–8%) makes it one of the most important precancerous lesions of the oral cavity.[78] A recent case-control study in India found that development of OSF amongst non-smokers and non-drinkers was significantly associated with chewing betel quid without tobacco (adjusted OR 56.2, 95%, CI 21.8–144.8).[79] This association suggests that the carcinogenesis related to the chewing of betel quid, occurs early in the head and neck cancer progression model.

As for the likely mechanism of carcinogenesis, areca nut extract has been shown to inhibit the growth of oral mucosal fibroblasts and keratinocytes by inducing cell-cycle dysregulation.[80] Several of the areca nut alkaloids (e.g., arecoline) and their nitrosated derivatives have been implicated, in particular 3-(*N*-nitrosomethylamino) propionaldehyde (NMPA) is highly cytotoxic and genotoxic to cultured human buccal epithelial cells, decreasing cell survival in a dose-dependent manner and forming DNA single strand breaks and DNA protein cross-links.[81]

The contribution of persistent oral keratinocyte inflammation through impairment of T-cell activation, and induction of local cytokine production (prostaglandin E2, TNF-α and interleukin-6) by areca nut extract, appears to be important;[82] while other work has implicated the production of reactive oxygen species (ROS) together with local lime-induced cell proliferation as another possible mechanism.[83]

## Marijuana

As with betel quid, evidence that marijuana is an independent risk factor for HNSCC has been difficult to establish because most users are often heavy users of both tobacco and alcohol. One small hospital-based case-control study has demonstrated an OR of 2.6 (95% CI 1.1–6.6) for HNSCC, adjusted for cigarettes, with some suggestion of a dose–response relationship.[84] This is in keeping with the finding that marijuana smoke has four times higher tar levels compared to tobacco and higher concentrations of aromatic hydrocarbons and benzopyrene. However, a recent large population-based case-control study looking only at OSCC, has not confirmed this association.[85] The fact that typical marijuana smokers are not long-term users, and that marijuana is not consumed at the same level as tobacco by a typical cigarette user, probably explains this difference. The picture may become clearer as the prevalence of long-term marijuana use increases in the population.[86]

# OCCUPATIONAL EXPOSURES

It is thought that occupational exposure has a small but definite role in the development of HNSCC. The one exception to this is sinonasal malignancy in which occupational exposure is a major determinant of disease. In general, HNSCC has its highest prevalence in unskilled manual workers who, as well as being exposed to the greatest levels of occupational substances, are also heavy smokers and drinkers. As with betel quid and marijuana, establishing a definite link is difficult because of confounding. It is likely however that at least 10% of head and neck cancer is related to occupational exposure; the most important occurring in the leather, textile, metal and woodworking industries.[87] Table 3.4 lists the major substances which have been implicated. The evidence is generally better for specific agents (e.g., chromium), which themselves may be responsible for the carcinogenicity of less specific agents (e.g., paint).

## WOODWORKING AND CARPENTRY

Exposure to wood dust (especially hardwood) is the most important occupational risk factor for adenocarcinoma of the nasal cavity and paranasal sinuses.[88] It is also associated with squamous cell carcinoma of this region.[89] Wood dust exposure has been implicated in SCC of the larynx with a risk of 2.5–8.1 in woodworkers and furniture-makers (adjusted for smoking and alcohol

*Table 3.4 Occupational factors implicated in the etiology of head and neck cancer*

| Strong Evidence | Weak Evidence |
|---|---|
| Arsenic | Asbestos |
| Cement / Stone dust | Bis-chloroethyl ether |
| Chromate | Coal dust |
| Formaldehyde | Dyes |
| Nickel compounds / alloys | Synthetic mineral vitreous fibres |
| Polycyclic aromatic hydrocarbons (PAH) | Pesticides |
| Radium | Diesel/petrol exhaust (containing PAH) |
| Vinyl chloride | Metal working fluids |
| Wood dust | Mustard gas |
| | Oil/grease (containing PAH) |
| | Solvents/paints (containing chromium) |
| | Rubber/bitumen |
| | Sulphuric acid mist |

intake), but this maybe related to their other exposures which could not be controlled such as formaldehyde, inorganic arsenic, chromates and phenols.[90]

### LEATHER AND TEXTILE INDUSTRY

Several case-control studies have suggested an elevated risk of oral, pharyngeal and laryngeal cancer in textile and leather workers.[91,92] The carcinogens that are likely to be responsible for this increase in risk include hexavalent chromium, azo and benzidine-based dyes.[93]

### METAL WORKING

Associations with laryngeal and pharyngeal cancer have been found in metal workers (e.g., blasters, cutters, welders, pipe fitters, plumbers and boiler makers) with risk estimates varying from 1.5–7.4 after adjustment for smoking and alcohol.[94] The implicated carcinogens in this industry include nickel, chromium and polycyclic aromatic hydrocarbons (PAH).

### BUILDING AND CONSTRUCTION INDUSTRY

Builders and construction workers are exposed to variable amounts of cement and stone dust. Cement dust in particular is associated with development of laryngeal SCC at the highest level of exposure after adjusting for tobacco use[95] and appears to be associated with other UADT sites.[96]

Asbestos exposure is associated with development of lung cancer and mesothelioma and the IARC has declared it as a known carcinogen. However, a link with laryngeal cancer remains unproven.[97]

There is some evidence that workers employed in road paving, asphalt mixing and other jobs entailing exposure to bitumen fume might be at increased risk of HNSCC when compared to workers in ground and building construction, but again there is confounding from other exposures and lifestyles.[98]

### OTHER SPECIFIC EXPOSURES

Nickel is associated with laryngeal carcinoma in nickel refiners[99] and also cancer of the major salivary glands,[100] while vinyl chloride, which is another known human carcinogen, is associated with an elevated risk of oral cavity and pharyngeal cancers in workers who are involved in its manufacture.[101]

## GASTROESOPHAGEAL REFLUX

The role of gastroesophageal reflux disease (GORD) in the development of upper aero-digestive tract carcinoma has been the subject of much discussion recently. While it is clear that reflux can cause local irritation and mucosal damage in the laryngopharynx resulting in, for example, vocal cord granulomas, it has been difficult to prove that it is associated with cancer development. The association is plausible given the link between reflux esophagitis, Barrett's metaplasia and subsequent development of esophageal carcinoma and also the inflammation from poor oral hygiene, which can result in oral cancer.

Interest was first directed at laryngeal carcinoma occurring in non-smokers and non-drinkers, where reflux was identified as the only risk factor.[102] It may also be associated with smoking and alcohol carcinogenesis, since tobacco causes a decreased lower esophageal sphincter pressure and increased acid secretion[103] while

alcohol reduces gastric emptying and alters oesophageal motility.[104]

A large case-control study of laryngeal and pharyngeal squamous cell cancers has shown that GORD is associated with development of both, with an adjusted odds ratio of 2.3 [95% CI 2.1–2.5] for laryngeal cancer and 1.9 [1.7–2.2] for pharyngeal cancer (outpatients figures adjusted for age, gender, ethnicity, smoking and alcohol).[105] Furthermore, a prospective study has demonstrated some evidence of a dose–response element to the association with an increase in the degree of reflux in patients with laryngeal cancer compared to a control group with laryngitis. Finally, a mechanism of dysplasia and carcinoma development has been proposed using the hamster cheek pouch model, suggesting that reflux acid may act as a tumor promoter like alcohol and that it may act synergistically with tobacco.[106]

So, although it is likely that GORD plays only a small part in the development of laryngeal cancer, it is significant because with the evolution of reflux treatment, any local laryngopharyngeal inflammation is easily reversed. It is not clear whether it is linked with other sites such as the hypopharynx and most importantly, whether screening and treating those at risk has any impact on reducing cancer incidence.

## DIETARY FACTORS

Dietary factors have a small, but nevertheless important role in the development of head and neck cancer. The relationship is extremely complicated however, with methods of food preparation and ingestion being implicated, as well as excess or insufficient intake of various components.

### FOOD

The major vitamin deficiencies linked with increased risk of HNSCC include that of vitamins A, C, E, beta carotene and riboflavin. Deficiency of iron, zinc and selenium also seem to be associated.[107] Accordingly, case-control studies consistently report the protective effect associated with increased fruit and vegetable intake. In western countries in particular, it is these nutrients that are often missing in the diet of chronic alcoholics.

Tomatoes in particular seem to have a significant protective effect. A case-control study in Uruguay found that tomato consumption was associated with a decrease in risk of UADT cancer (adjusted OR 0.30, 95% CI 0.18–0.51).[108] They contain high levels of micronutrients such as lycopene, vitamin C, folate, phytosterol and flavonoids.

A further component of fruit and vegetables that is likely to be beneficial is fibre. Another study found a strong inverse association between fibre intake and risk of laryngeal cancer.[109]

Decreased meat and fat consumption seems to be associated with reduced risk of cancer.[110] However, this is may not be the case in all populations. A study in female tobacco- and betel quid-chewers in south India, found that a diet deficient in foods of animal origin was the most significant risk factor for oral pre-malignancy rather than a diet deficient in fruits and vegetables.[111]

One specific association to consider is the link between traditional salted fish and nasopharyngeal carcinoma in the southern Chinese population. The carcinogens in this case are thought to be *N*-nitroso compounds such as nitrosamine. This relationship has not been demonstrated in other populations. For example, a large cohort study in Finland found no association between head and neck cancer and salted or smoked fish.[112] However, a case-control study in Uruguay, did find an association between salted meat consumption and laryngeal carcinoma.[113]

### BEVERAGES

#### Mate

Mate is a beverage like tea, popularly consumed in South America. It is brewed from the dried leaves of the perennial tree *Ilex paraguariensis* (yerba mate) which belongs to the *Aquifoliaceae* family.[114] It has been found to be associated with a three-fold increase in risk of laryngeal cancer, after controlling for the effects of age and tobacco and alcohol consumption.[115] It also appears to be associated with oral and pharyngeal cancer.[116]

### SPECIFIC CONSIDERATIONS

#### Iron-deficiency anemia

The association of Plummer–Vinson or Patterson–Brown–Kelly syndrome (an upper esophageal web with iron deficiency, koilonychia, glossitis and angular stomatitis) with post-cricoid carcinoma, is important to mention, since it is the only subsite of the head and neck where the incidence is higher in women.

# Viral Oncogenesis

It is possible that up to one-fifth of human cancers may be associated with viral infection. This includes proven associations such as carcinoma of the cervix (human papillomavirus) and hepatocellular carcinoma (hepatitis C virus). Viral oncogenesis was first demonstrated

in the 1930s when cotton-tailed rabbit papillomas were inoculated into a domestic rabbit to induce papillomas, a few of which became skin carcinomas. However, it was not until 1964 that the first association between viral infection and human cancer was made by Epstein and Barr in a cell line isolated from a case of Burkitt's lymphoma.[117]

## EPSTEIN–BARR VIRUS

Epstein–Barr virus (EBV) is a member of the herpesviridae family. It is a double-stranded DNA virus which infects B lymphocytes and occasionally, squamous epithelial cells of the oropharynx. The virus is extremely common, with over 90% of the worldwide adult population being asymptomatic, healthy carriers. Primary infection normally occurs in early childhood and a latency resides in a small number of memory B-cells.[118] The usual source of transmission is by saliva through aerosol or direct contact.

The possibility of the virus being associated with nasopharyngeal carcinoma (NPC) was first suggested because of the presence of EBV antibodies.[119] The association is now conclusive, since the virus is consistently detected by polymerase chain reaction (PCR) in all cases of undifferentiated SCC (World Health Organization [WHO] type III) and moderately differentiated SCC (type II), and also well differentiated (type I) cases in endemic regions. The exception seems to be type I tumors in non-endemic areas which probably result from smoking and alcohol use.[120] However, whilst it is accepted that EBV is necessary for the development of nasopharyngeal carcinoma, it is probably not sufficient on its own to cause cancer.

The highest incidence of nasopharyngeal carcinoma is found in southern China where epidemiological studies suggest that important environmental cofactors associated with the Chinese way of life play a role. These include traditional salt-cured fish which contain carcinogenic nitrosamines and herbal medicines taken as snuff, which contain tumor-promoting substances of phorbol ester type. There is also a genetic predisposition since those with certain specific major histocompatibility complex class I profiles, including the HLA-A2 allele and haplotypes Aw19, Bw46 and B17 show a higher incidence of nasopharyngeal carcinoma.[121]

## HUMAN PAPILLOMAVIRUS

The human papillomavirus (HPV) is a small, double-stranded DNA virus belonging to the papavoviridae family, which replicates within epithelial cells of the host's mucosa and skin. HPV infection is the commonest sexually transmitted disease worldwide with prevalence rates in sexually active women ranging from 10%–50%.[122] The virus normally results in benign, self-limiting warts or tumors, characterized by abnormal maturation and differentiation of epithelial cells. After initial infection, the virus can remain latent in the basal layer of the epithelium for months or even years before histological change is detected.[123]

Epidemiological studies have established that it is the central cause of invasive cervical carcinoma, with HPV DNA being demonstrated in almost all (99.7%) of tumor biopsies in a study coordinated by the International Agency for Research on Cancer (IARC).[124] There are approximately 100 different subtypes now recognised, with at least 14 of these being referred to as high-risk because they are significantly associated with progression to invasive cervical cancer.[125] By far the commonest subtype is HPV-16, which was found in 51% of cases in a recent meta-analysis.[126]

The HPV genome consists of three regions: an upstream regulatory protein and two regions named according to the phase of infection in which they are expressed, the early (E) and the late (L) regions. The most potent oncogenes, E6 and E7, code for proteins that facilitate the proliferation of infected cells by disrupting the function of the tumor suppressor proteins p53 and pRb (retinoblastoma protein) respectively. This can lead to immortalization of the cell and eventually to carcinogenesis. The relation between this and HPV is that HPV-positive cancers are more likely to express wild-type p53 than those lacking HPV DNA, although there are small numbers of tumors with both the mutation and the virus.

A link between HPV and HNSCC was first suggested more than 20 years ago.[127] Since then, HPV DNA has been isolated from tumors throughout the upper aero-digestive tract, with a wide variation in prevalence. Evidence from several case series suggests that there is a stronger link between HPV and oropharyngeal squamous cell carcinoma (OPSCC) than other head & neck sites. In particular, one study found that HPV was nine times more likely to be found in squamous cell carcinoma of the tonsil (TSCC) compared to other HNSCC.[128] The possible role of HPV in the development of oropharyngeal tumors is further supported by the findings that patients with a history of anogenital cancer have a fourfold risk of TSCC than the general population;[129] and that husbands of women with cervical cancer are twice as likely to get tonsillar or tongue carcinoma.[130]

In the multicentre IARC HPV and oral cancer study,

HPV DNA was detected in 18.3% of oropharyngeal SCC compared to 3.9% of oral cavity SCC. The presence of antibodies to HPV16 E6 or E7 were associated with increased risk of cancer of both sites: oral cavity OR = 2.9 (95% 1.7–4.8) and oropharynx OR = 9.2 (95% 4.8–17.7).[131] A small but significant association has also been demonstrated for HPV16 exposure and laryngeal SCC (OR 2.6, 95% CI 1.4–4.9).[132]

Typically, HPV-positive HNSCCs are poorly differentiated and basaloid in appearance, present in a younger age group with a more advanced stage, but show improved survival. It appears that HPV-positive oropharyngeal and tonsillar carcinomas may even be different tumor entities which are less dependent on traditional risk factors such as smoking and alcohol and demonstrate a more favourable prognosis.[133] The final confirmation of this possible etiology lies with the future use of HPV vaccination, the first trials of which have already shown encouraging reductions in cervical intraepithelial neoplasia related to HPV 16.[134]

### HUMAN IMMUNODEFICIENCY VIRUS

The HIV virus has a strong association with cancer. Of the three cancers considered AIDS-defining, two can occur in the head and neck region—Kaposi sarcoma (KS) and non-Hodgkin lymphoma (NHL). Both of these are themselves associated with specific viral infections: KS with human herpes virus 8 (HHV 8) and NHL with Epstein–Barr virus. Hodgkin lymphoma is also more prevalent in HIV patients, however, the disease appears to be different to that of the general population. In particular, HIV-associated Hodgkin disease is positive for Epstein–Barr virus in 80%–100% cases compared to 40% of normal Hodgkin lymphoma.

### HUMAN HERPES VIRUS 8

This herpes virus was first identified in 1994 following earlier suspicions that HIV-associated KS lesions were associated with an infectious etiology (because of the higher incidence in homosexual males). Subsequently, HHV 8 has been found in over 95% of KS whether related to HIV infection or not.

## Radiation

### IONISING RADIATION

Certain specific head and neck cancers are associated with radiation exposure. This can occur from environmental sources, occupational exposure or through therapeutic radiation. Radiotherapy in particular is associated with thyroid and salivary gland carcinomas and sarcomas of the head and neck. However, despite the large numbers of patients treated by this modality, radiotherapy-induced tumors remain uncommon.[135] In a case-control study, prior radiotherapy of the head and neck was associated with development of thyroid cancer (OR = 2.8, 95% CI 1.2–6.9).[136] Risk was inversely related to age at irradiation and was highest among children exposed under age 10. An important point in this study was the finding that there was no association with diagnostic x-rays, radioactive isotope scans or occupational radiation exposure. Treatment with radioactive iodine also does not seem to be associated with later thyroid cancer development. Another study did find an association with occupational radiation exposure and development of salivary gland tumors.[100]

Environmental exposure includes natural background radiation (e.g., radon) or fallout from nuclear reactor accidents (e.g., Chernobyl), or the atomic bomb explosions in Japan. For example, 6.7% of the survivors of Hiroshima and Nagasaki developed papillary thyroid cancers, far higher than that expected in the general population. There was no evidence of an increase in HNSCC, however.[137]

The current low incidence of radiation-induced head and neck cancers is likely to rise in the future as cancer survival improves and life expectancy continues to increase.[138]

### SUN EXPOSURE

Exposure to ultraviolet radiation is associated with squamous cell carcinomas of the skin in the head and neck region, and includes sites such as the lip.[139] This is particularly relevant in equatorial and tropical countries like Australia where a large proportion of the population is not indigenous. Occupations that work outside are at an increased risk. For example, a study in northern France found that farming was associated with development of lip cancer (OR 5.3, 95% CI 1.1–26.8).[140]

### SUMMARY

Head and neck cancers are a diverse group of tumors with multiple causation and show extensive variation in international incidence. Although some genetic predisposition to the disease exists, the main etiology lies in exposure to environmental carcinogens. The major worldwide carcinogen is tobacco, with alcohol acting as a significant co-carcinogen and promoter. The consumption of betel quid is an additional risk factor in developing countries. A decrease in global tobacco and alcohol consumption will almost certainly result in reduction of this disease.

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