2. BACKGROUND
2.0 BACKGROUND

The International Classification of Diseases defines Head and Neck cancers as cancers of the oral cavity and pharynx (ICD-10, C00.0-14.0) and oral cavity cancers include cancers of the tongue, mouth, gum, floor of the mouth, palate and other and unspecified parts of the mouth (ICD-10, C01.0-06.0). Malignancies arising from the mucosa of the oral cavity are epithelial in origin and are, therefore, classified as squamous cell carcinomas. Oral cancers have much in common with squamous cell carcinomas arising elsewhere in the upper aero digestive tract, sharing common risk factors. Hence studies of head and neck cancer are frequently referred when issues relevant to oral cancer are discussed. Squamous cell carcinoma account for more than 90% of all oral cancers (Zarbo 1988; Silverman 1990). According to the degree of differentiation, three subtypes are defined: (1) well-differentiated squamous cell carcinoma showing more than 75% keratinization; (2) moderately differentiated squamous cell carcinoma with 25-75% keratinization; and (3) poorly differentiated squamous cell carcinoma with less than 25% keratinization. The majority of cases are moderately differentiated carcinomas. The definitions used to categorize cases are “localized” referring to tumor limited to the primary site without known spread to lymph nodes or adjacent tissues; “regional” indicating the presence of invasion of surrounding tissues and/or involvement of lymph nodes; and “distant metastasis” indicating the spread to distant organs.

Oral and pharyngeal cancer is the sixth most common cancer reported globally with the annual incidence of over 300,000 cases, of which 62% arise in developing countries (Parkin 1988). It is very common in countries like India, Pakistan, Taiwan and some areas of France and more common among males than among females. It is predominantly a disease of people over 40 years. In high prevalence areas many patients are less than 40 years old owing to the prevalence of various risk habits. For the past 2-3 decades a rise in the incidence of oral cancer among young adults have been observed (Cusumano 1988; McGregor 1989; Atula 1996; Myers 2000). In industrialized countries men are affected twice or thrice as common as women. But in high incidence countries like India, the incidence of tongue and other intraoral cancer for women is greater than or equal to that of men (Vargas 2000). The incidence in India is one of the highest in the world with the age-adjusted rates of 17.7/100000 among males and 9.3/100000 among females. It constitutes 12% of all cancers in men and 8% of all cancers among women. It has been
estimated that 83000 new oral cancer cases and 46000 deaths occur annually in India (Parkin 2002; Ferlay 2004).

The survival of oral cancers has not significantly improved for the past few decades, the reasons being presentation of cases in the advanced stages and the occurrence of second primaries. Nearly two thirds of all oral cancers are diagnosed only after they become locally advanced (Barry 1989; Vokes 1993; Forastiere 2001).

In a study on survival from head and neck cancers from India (Yeole 2000), it was found that the 5-year age standardized relative survival of head and neck cancers was 35.8. There was three-fold increased risk of death in patients with regional spread and six-fold risk in those with distant metastasis. Less than one fourth of cancers were localized at the time of diagnosis. The five-year survival for localized cancers ranged from 52.9% (oropharynx) to 80.2% (lip) depending on the subsite. Factors like age, socio economic indicators, site of origin and clinical extent of disease influence the variations in survival.

Oral cancers are primarily treated by surgery or radiotherapy or a combination of both. Detection of oral cancers in the early stages leads to reduction in mortality. Improved survival can be achieved by early detection and adequate loco regional treatment.

2.1. Oral Carcinogenesis

Oral cancer arises from a premalignant stage, which may be clinically obvious as leukoplakia, or it could be clinically occult; followed by outgrowth of clonal populations associated with cumulative genetic alterations and phenotypic progression to form invasive malignancy. This carcinogenesis process is both a multi-step and multi-stage process.

2.1.1 Multi step carcinogenesis

Cancer development is a multi step process in which exposure to a carcinogen (eg. cigarette smoke) results in repeated damage and repair until the accumulated exposure triggers a transformation from normal to premalignant cells (i.e from normal cells to metaplasia and dysplasia) and eventually to carcinoma. Normal cells are not neoplastically transformed by a single oncogene but rather require two or more cooperating oncogenes and inactivation of multiple tumor-suppressor genes supporting the multistep or multi-hit model of carcinogenesis. Recent evidence suggests that 3-10 somatic mutations are needed to convert a normal cell to a malignant cell in humans. Analysis of multistep carcinogenesis at the molecular level indicates that the process of
neoplastic evolution is significantly more complicated than the relatively simple two-stage (initiation and promotion) model of carcinogenesis or even a three-stage model of initiation, promotion, and progression. An example of multistep carcinogenesis on the molecular level has been best described by Vogelstein and colleagues in colon carcinoma. The model shows that multiple genetic changes must occur after the promotion or clonal growth of the initiated cells. Thus, the progression phase of carcinogenesis represents multiple stages at which chemicals might influence the neoplastic process. There are three general mechanisms by which a substance can influence the multistep carcinogenic process: i) by inducing heritable mutation in a critical gene, ii) by inducing heritable, epigenetic change in a critical gene, iii) by increasing clonal expansion of a cell with a heritable alteration in a critical gene, allowing for increased probability of additional events.

Figure 2.1: Histological progression model of oral squamous cell carcinoma

The histological progression model for oral Squamous cell carcinoma (SCC) is well established. It is generally believed to develop through sequential stages of premalignant /pre-invasive lesions: hyperplasia, mild, moderate, severe dysplasia, carcinoma in situ (CIS), and finally invasive SCC. Califano and his colleagues were the first to develop a molecular progression model for oral carcinogenesis, using findings from microsatellite analysis of archival tissue (Califano 1996). Eighty-seven lesions of the head and neck-preinvasive lesions and benign lesions associated with carcinogen exposure, were tested using microsatellite analysis for allelic loss at 10 major chromosomal loci -9p21, 3p21, 17q13, 11q13, 13q21, 14q 31-32.1, 6p, 8q, 8p, 4q 26-28 which have been defined previously. In this model, allelic loss has been used as a molecular marker for inactivation of putative tumor suppressor genes. Allelic imbalance may also occur from oncogene amplification (e.g., cyclin D1 on 11q13). In the case of Head and Neck Squamous Cell Carcinoma (HNSCC), chromosomal loss and amplification at the 10 loci have been
confirmed by other complementary methods. This model supports the initial observations of the colorectal molecular progression model proposed by Fearon and Vogelstein (Vogelstein 1988), in that (a) both oncogenes and tumor suppressor genes are involved in tumor progression; (b) specific events in head and neck cancer generally occur in a distinct order of progression, with loss at 9p21 or 3p among the earliest detectable events and (c) it is the accumulation and not necessarily the order of genetic events that determines progression.

They suggested that it is the accumulation of these genetic changes (number of changes or hits) that is most strongly associated with risk of progression to cancer, referred to as the process of multistep carcinogenesis.

2.1.2 Field Cancerisation

Head and neck cancer patients often present with premalignant lesions and Multiple Primary Tumors (MPTs) in their Upper Aerodigestive tract (UADT). This led Slaughter et al. in 1953 to postulate the concept of field cancerization. Head and Neck Squamous cell carcinoma (HNSCC) results from a multistep carcinogenesis process, which occurs over large areas of the upper aerodigestive tract epithelium exposed to carcinogens. This condemned mucosa contains multiple transformed clones that can develop into new primary tumors at a rate of 30% over five years. This process is called “field cancerization” (Slaughter 1953). It could be the result of either independent molecular events affecting multiple cells separately or as molecular event in a single clonal progenitor that gives rise to this phenomenon, via mechanisms of widespread clonal expansion or an alternative means of undergoing lateral spread across the mucosa of the upper aerodigestive tract.

![Figure 2.2: Genetic Explanation of Slaughter’s Concept of Field Cancerization (Boudewijn 2004)](image-url)
Initially a “patch”, a clonal unit of mutated cells (a stem cell and daughter cells) develops which is converted into a “field” - an epithelial lesion consisting of cells with cancer related genetic alterations expanding at the expense of normal tissue. During field progression a number of genetic alterations take place. Three mechanisms have been proposed for the lateral spread of “patch” to form a “field” (i) lateral spread of transformed cells along the basement membrane, (ii) implantation of the transformed cells to a distant site within the oral cavity and (iii) development of a new clone away from the primary transformed site. Based on these mechanisms the clones within the field may be same (mechanisms i, or ii) or different (mechanism iii) (Boudewijn 2004).

2.2 Genetic Alterations

The genetic alterations include gene amplification, over expression of oncogenes such as MYC, ERBB-2, EGFR, RB, RASSF1A, FHIT, CCND1 and mutations, deletions and hypermethylation leading to $p16$ and $p53$ TSG inactivation. And loss of heterozygosity in several chromosomal regions is frequently observed, suggesting that other tumor suppressor genes not yet identified could be involved in the tumorigenic process of head and neck cancers. Genetic polymorphisms of carcinogen metabolizing genes or DNA repair genes influence susceptibility of individuals to cancer development. The exact temporal sequence of the genetic alterations during head and neck squamous cell carcinoma (HNSCC) development and progression has not yet been defined and their diagnostic or prognostic significance is controversial.

2.2.1 Loss of Heterozygosity (LOH)

Deletion of specific chromosomal regions is one of the most common genetic events observed in solid tumors and the deleted regions are believed to contain potential tumor suppressor genes.

LOH is defined as a loss of genomic material (from a few thousand to a whole chromosome) in one of a pair of chromosomes. It represents the loss of a single parent's contribution to part of the cell's genome. It can arise by two methods: i) a region of a chromosome is deleted, resulting in only one copy remaining, ii) genetic recombination leaves the cell with two copies of the chromosomal region, but both coming from the same parent. The highest LOH frequencies occur on chromosomal arms 3p, 9p and 17p (Maestro 1993; Ah-See 1994), which harbor unknown and already cloned tumor suppressor genes, such as VHL (3p), $p16$ (9p) and TP53 (17p).
It is suggested that LOH at 9p is the earliest event, associated with transition from normal to benign hyperplasia; allelic losses in the 9p21 region, possibly associated with the genes encoding the p16 and p14 cyclin dependent kinase inhibitors are present in premalignant lesions and oral cancer. LOH at 3p and 17p were more often associated with dysplasia, whereas CIS and SCC were characterized by additional deletions on 4q, 6p, 8, 11q, 13q, and 14q (Califano 1996).

Virtually all-progressing lesions (97%) had LOH at 3p and/or 9p, suggesting that loss in these arms is a prerequisite for progression, although such loss alone is probably insufficient for malignant transformation. Additional loss at other chromosome arms significantly increased the cancer risk. Based on these results, three risk groups were defined: a low-risk LOH pattern (retention of 3p and 9p); an intermediate-risk (LOH at 3p and/or 9p) and a high-risk pattern (LOH at 3p and/or 9p plus loss at 4q, 8p, 11q, 13q or 17p). Lesions with the high-risk pattern had a 33-fold increase in cancer risk as compared with those with a low-risk pattern (Mao 2000; Rosin 2000).

### 2.2.2 Oncogene activation

Mutation of *H-ras* oncogene is less common in Western countries (<5%) (Yarbrough 1994). In India, a high incidence of about 35% has been reported in OSCC in which betel-quid and reverse smoking are probable initiators (Saranth 1991; Field 1992). Yet another study has shown significant risk (odds ratio 1.6) associated with an H-ras gene polymorphism in the Indian population (Sathyan 2006). A nodal example is the ras gene family that includes the H-, K- and N-ras oncogenes. Indeed, constitutive activation of the K-ras protein in a mouse model is sufficient to induce oral tumor formation.

Amplification of the 11q13 region is frequently observed in head and neck tumors. Several putative oncogenes have been identified on 11q13, including *bcl-1*, *int-2*, *hst-1*, *EMS-1* and cyclin D1/PRAD-1. Amplification and co-amplification of *bcl-1*, *int-2* and *hst-1* have been found in 30-52% of the head and neck tumors (Berenson 1989; Muller 1997). Cyclin D1 overexpression has been described in 39-64% of primary HNSCC (Åkervall 1997; Michalides 1997). In another study of HNSCC, overexpression of cyclin D1 has been correlated with lymph node metastases and advanced clinical stage implicating poor prognosis (Fracchiola 1997).
Amplification and over expression of the oncogenes MYC, ERBB-2 and epidermal growth factor receptor (EGFR) have been observed in HNSCC, and correlated with a poor prognosis.

The members of the MYC gene family (c-myc, N-myc, L-myc) encode for a 62-kDa protein with transactivational activity. The MYC proteins (p62myc) are able to dimerize with a second protein, max. Over expression of MYC leads to increased amounts of myc-max heterodimers and reduced maxmad heterodimers, changing the regulation of many genes and contributing to malignant transformation. The frequency of c-myc amplification and over-expression varies from 9 to 48% (Rodrigo 1996). Porter et al demonstrated that the prognosis was worse for patients with c-myc amplification (Porter 1994).

ERBB-2 gene (also named NEU or HER-2) encodes for a 185-kDa protein with tyrosine kinase activity, homologous to the EGFR (Schecter 1984). In head and neck carcinomas, the frequencies of ERBB-2 amplification and over-expression range from 0 to 41%. The prognostic value of these genetic alterations observed in ERBB-2 oncogene is controversial.

EGFR is a transmembrane tyrosine kinase receptor of the erbB-family that is normally expressed at low levels on the surface of most normal cells. EGFR is over expressed in head and neck tumors. Over expression of EGFR has been associated with a more aggressive clinical behavior, resistance to treatment and a poor prognosis (Hendler 1988; Miyaguchi 1990). Studies have shown EGFR over expression as an independent prognostic marker of survival in betel quid chewers and a component of a prognostically significant molecular profile (Chen 2003). Signal transduction from activated transmembrane receptors like EGFR depends on a variety of downstream mediators that are frequently altered in various cancer types.

2.2.3 Tumor suppressor gene inactivation

2.2.3.1. P16

The p16 tumor suppressor gene located on chromosome 9p21 encodes a 16-kDa protein that belongs to an important group of cyclin-dependent kinase inhibitors (CDKIs), which includes p15INK4b, p21WAF1 and p27KIP1. These genes regulate the G1 phase of the cell cycle in a negative way. The p16 gene product binds to CDK4 and CDK6 inhibiting their association with cyclin D1. The inhibition of the cyclin D1/CDK4/6 complex
activity prevents pRB phosphorylation and the release of E2F, leading to inhibition of the cell cycle in the G1-S transition. Genetic abnormalities inactivating the p16 gene might confer cell growth advantages contributing to the tumorigenic process. High frequencies of loss of heterozygosity on the short arm of chromosome 9 (9p21-22), where the tumor suppressor gene p16 is located, have been reported in head and neck tumors, including dysplasia and carcinoma in situ, suggesting the involvement of this region in the early stages of the disease.

The high incidence of p16 inactivation in head and neck tumors (20-67%) indicates that this gene plays an important role in the development of the disease. Homozygous deletions and hypermethylation are considered to be the major genetic mechanisms for p16 inactivation.

2.2.3.2 TP53

Genetic alterations leading to loss of normal function of the TP53 tumor suppressor gene is believed to contribute to the development of the majority of human cancers. The TP53 gene is located on chromosome 17p13.1 and consists of 11 exons coding for a nuclear phosphoprotein, which can bind to specific DNA sequences acting as a transcription factor. Most TP53 gene mutations are missense mutations that tend to cluster within exons 5 to 8, spanning the evolutionarily conserved region of the protein.

The p53 expression may predict poor prognosis in the subset of patients with low stage, node negative disease or in those carrying specific TP53 mutations. Tumors with TP53 mutations seem to be more resistant to radiotherapy.

In a study by Saranath et al on p53 inactivation in chewing tobacco-induced oral cancers and leukoplaikias, it was found that the p53 alterations were present in 46% of oral cancer tissues and 27% of potentially malignant oral leukoplaikias suggesting a critical role for p53 gene in a significant proportion of oral cancers in India (Saranath 1999). The p53 alterations may be useful early biomarkers in chewing tobacco-associated oral malignancies.

2.2.4 Other genes

1.2.4.1 Matrix metalloproteinases

Matrix metalloproteinases (MMP) are zinc metalloenzymes with the ability to degrade the components of the ECM (extracellular matrix). Their action is crucial during the
progression of cancer since they allow the remodeling of the surrounding healthy tissues and enable local invasion. It has been demonstrated that gelatinases (MMP-2 and -9), stromelysins (MMP-3, -10 and -11), collagenases (MMP-1 and -13) and membrane-bound MMPs (MT1-MMP) are expressed in OSCC and may play a role in its progression. MMP-2 expression is shown to be more prominent than MMP-9 in OSCC samples and correlated with lymph node metastasis. The association between the overexpression of MMP-2 and MMP-9 and alcohol consumption, suggest that the contribution of alcohol in the carcinogenetic process of OSCC may be attributed to the overexpression of these two enzymes.

2.2.4.2 Nuclear Factor kappa B (NFkB)

NFkB regulates several genes that mediate tumorigenesis and metastasis. It is a heterotrimeric complex consisting of p50, p65 and IKBA present in the cytoplasm in the inactive state. It is activated by carcinogens, tumor promoters, inflammatory cytokines and by chemotherapeutic agents (Pahl 1999). Activation of NFkB has been implicated in cellular transformation, tumor promotion, angiogenesis, inflammation, invasion and metastasis (Garg 2002). NFkB activation can suppress apoptosis.

2.2.4.3 Cyclo-oxygeneases

COX-1 primarily regulates housekeeping functions such as in gastrointestinal mucosal cells and platelet function. COX-2 is the inducible form that is likely responsible for inflammatory and carcinogenic effects. Levels of COX-2 are increased in HNSCC as well as in normal-appearing mucosa adjacent to HNSCC, suggesting that increase of COX-2 expression can occur early during the head and neck tumorigenesis (Chan 1999).

2.3 Risk factors/Etiological agents

Several etiologic agents are involved in the development of Head and neck cancers including oral cancers. They include tobacco and alcohol consumption, diet, genetic susceptibility, certain chemicals, and radiations in addition to viral infections such as exposure to human papilloma virus (HPV).

Tobacco and alcohol usage are the major risk factors, which lead to nutritional deficiencies, and susceptibility to various carcinogens and thus lead to immune suppression. Seventy five percent of oral cancers are attributed to tobacco and alcohol usage. Heavy alcohol drinkers are frequently heavy smokers as well (WHO. 1984; Gupta 1996; Yeole 1999; Gupta 2000). The risk for development of oral cancer is 3 to 9 times
greater in those who smoke or drink and as much as 100 times greater in those who both
smoke and drink heavily than in those who neither smoke nor drink (Neville 2002). A
wide variety of tobacco habits like bidi smoking, tobacco chewing, and cigarette
smoking, in that order, account for a large majority of these cancers (Sanghvi 1989).

2.3.1 Tobacco

WHO estimate has shown that 91% of oral cancers are due to tobacco usage (WHO.
1995).

Chewing (Smokeless tobacco)

Some of the common forms of smokeless tobacco used in India are pan, betel quid, khaini
(tobacco & lime), mishri (burned tobacco), zarda (boiled tobacco), gadakhu (tobacco &
molasses), mawa (tobacco, lime & areca)

Chewing of pan with or without tobacco is an independent risk factor (Muwonge 2008).
Paan represents a cheap pharmacologically addicting stimulant, used by people within the
lower socio economic class, in South Asia. Betel quid is a combination of the betel leaf
(Piper betel), areca nut (Areca catechu), slaked lime (Calcium hydroxide) and catechu
(Acacia catechu). Studies from India, Taiwan, South Africa showed that the use of
arecanut alone without tobacco was associated with oral precancerous and cancerous
lesions (Ko 1992; van Wyk 1993).

In a meta analysis of 17 published studies by Thomas and Wilson, the carcinogenicity of
pan/betel quid is well established with a relative risk of around 10 (Thomas 1993). A
study from Pakistan showed an Odds Ratio (OR) of 12.5 for paan-tobacco chewing and of
5.2 for chewing paan without tobacco (Merchant 2000). In a case control study conducted
in Bhopal, it was found that there was a 6 fold increase in the risk for oral cavity cancer
among tobacco quid chewers and the population attributable risk percentage (PARP) was
66.1% (Dikshit 2000) Another case control study conducted in Kerala has shown that
among males, an adjusted RR of 6.14 was associated with chewing 10 or more pan-
tobacco quids per day relative to those who never chewed and the corresponding value in
female was 9.27 (Sankaranarayanan 1989).

In a case control study from South India to evaluate the influence of smoking, drinking,
paan chewing and oral hygiene on oral cancer, including 591 oral cavity cancer cases and
582 controls (Balaram 2002), it was found that an OR of 2.5 (95% CI 1.4-4.4) was found
in men for smoking ≥20 bidi or equivalents versus 0/day. The OR for alcohol drinking

14
was 2.2 (95% CI 1.4-3.3). The OR for paan chewing was more elevated among women (OR 42; 95% CI 24-76) than among men (OR 5.1; 95% CI 3.4-7.8). A similar OR was found among chewers of paan with (OR 6.1 in men and 46 in women) and without tobacco (OR 4.2 in men and 16.4 in women). Among men, 35% of oral cancer were attributable to the combination of smoking and alcohol drinking vs. 80% in the USA, Europe and Latin America (Blot 1988; Negri 1993; Fernandez-Garrote 2001) and 49% to pan-tobacco chewing. Among women, 95% of oral cancers were attributed to chewing and poor oral hygiene.

The use of betel quid, containing areca nut and tobacco, is associated with a much higher relative risk of oral cancer between 8-15 times as compared to that of 1-4 times, associated with using the quid without tobacco (Manjari 1999). Intensive health education programmes among 36000 betel quid chewers and smokers in India have shown a significant regression in precancerous lesions (Gupta 1986; Mehta 1986).

**Smoking**

In India, smoking of bidis, cigarettes, pipe, cigar, etc. is common. Over 300 carcinogens have been identified in tobacco smoke or its water-soluble compounds. The most studied are aromatic hydrocarbon benzopyrene and the tobacco specific nitrosamines (TSNs), N-nitrosonornicotine (NNN), N-nitrosopyrrolidine (NPYR), N-nitrosodimethylamine (NDMA) and 4-methylnitrosamino-1-3-pyridyl-1-butanone (NNK) (Hecht 1993). These agents act locally on keratinocyte stem cells and are absorbed. They produce DNA adducts, principally O-6 methyl guanine and these interfere with the accuracy of DNA replication leading to mutations which contribute to the molecular chain of events leading to malignant transformation of a cell and its clonal derivatives.

Contrary to the popular belief, bidi smoking is more hazardous than cigarette smoking (Dikshit 2000). Bidi is obtained by wrapping 0.2–0.3 g of tobacco in temburni leaf. One bidi is considered equivalent to one cigarette or one-quarter of a cigar. Studies have shown that bidis produce more carbon dioxide, nicotine, tar and alkaloids than regular cigarettes (Pakhale 1990; Pakhale 1998). Added to this, the filterless design of the bidi combined with low combustibility may contribute to higher toxin yields than with regular cigarettes (Pakhale 1990). And an adjusted Relative Risk (RR of 7.46) was found among males who smoked 20 or more bidis per day relative to non-smokers (Sankaranarayanan 1989).
A retrospective case-control study from Mumbai of male tongue cancer patients has shown that bidi smoking was a significant risk factor for BT and tobacco chewing for AT patients. And an excess risk of 45-79% was observed among alcohol drinkers (Rao 1998).

For smoking, previous studies showed that 70 to 90% of patients with leukoplakia were tobacco users and that 78% of leukoplakia lesions disappeared or regressed within 12 months after smoking cessation (Roed-Petersen 1971). A high frequency of potentially malignant dysplastic lesions and squamous cell carcinoma has been reported in reverse chutta smokers in India along the coastal districts of Vishakapatnam and Srikakulam (Gupta 1980) and in rural Andhra Pradesh (Daftary 1991; van der Eb 1993).

Studies have shown that there was a decrease in risk of oral and pharyngeal cancers as evidenced by the drop in the odds ratio relative to the duration since stopping. The risk of oral cancer associated with smoking is both dose and duration dependent while smoking cessation leads to a fall in risk (Blot 1988; Castellsague 2004; Rodriguez 2004). The excess risk of oral cancer from smoking almost disappears within 10 years of giving up, according to one review (International Agency for Research on Cancer 2004). However, since then a study showed that it takes more than 20 years for the risk to reduce to that of never smokers (Bosetti 2008).

### 2.3.2 Alcohol

Alcohol by itself is not a carcinogen (Doll 1981). Alcohol promotes carcinogenesis in a variety of ways. The proposed mechanisms are i) Acetaldehyde, which is the alcohol metabolite, has been identified recently as a tumor promoter (Blot 1992; Harty 1997), ii) Alcohol may act as a solvent and enhance the penetration of carcinogens into the target tissues (Stenback 1969), iii) nutritional deficiency associated with heavy drinking (Harris 1997), iv) alcoholic liver disease is common in heavy drinkers and that reduces the detoxification of active carcinogens (Kato 1994).

‘Toddy’ (a locally fermented distilled sap from palm trees), another locally brewed liquor called ‘arrack’ (approximately 40% ethanol) or Indian made foreign liquor (locally made liquor similar to that brewed in western countries) or combinations of at least two of the above types are the common forms of consumption.

Numerous studies have shown alcohol as a risk factor for oral cancers (Brugere 1986; Franceschi 1990; La Vecchia 1997). Some studies have shown that the consumption of alcohol was not related to leukoplakia (OR 0.76 (0.04–1.43) but to the malignant
transformation- odds ratio OR 2.37 (1.47–3.82). Smoking and betel quid are two risk factors associated with the occurrence of leukoplakia whereas alcohol was significantly responsible for malignant transformation (Shiu 2004).

Evaluation of the role of tobacco chewing, smoking and alcohol drinking patterns on the risk of cancer of the oral cavity in a nested case-control study suggested tobacco chewing as the strongest risk factor associated with oral cancer (Muwonge 2008). Also there exists a significant dose-response relation with the frequency and duration of chewing, smoking and alcohol drinking.

National Family Health Survey publishes data on the prevalence of habits in India (NFHS-3 2007). It was found that in India among males, 57.0% consumed tobacco in one form or the other, 32.7% smoked cigarettes/bidis, 31.9% consumed alcohol and among females, 10.8% consumed tobacco in one form or the other, 1.4% smoked cigarettes/bidis, 2.2% consumed alcohol. In Kerala among males, 43.5% consumed tobacco in one form or the other, 35.8% smoked cigarettes/bidis, 45.2% consumed alcohol and among females, 1.8% consumed tobacco in one form or the other, 0.1% smoked cigarettes/bidis, 0.7% consumed alcohol (NFHS-3 2007).

### 2.3.3 Genetic polymorphisms

Majority of drinkers and smokers do not develop cancers suggesting a genetic cause. Single nucleotide polymorphisms (SNPs) of genes coding for carcinogen metabolism enzymes or for DNA repair genes may explain individual differences in the susceptibility to carcinogens. These could include genes that may influence behavior, which might lead to increased alcohol or tobacco consumption, as well as phase I and phase II metabolizing genes (such as *ADH*, *ALDH*, *CYP*, *GST* and *N*-acetyl transferase genes) that are likely to be important in determining internal carcinogenic dose. The subsequent development of DNA mutations, repair of these errors, or cell apoptosis might also be regulated by DNA repair genes or tumor suppressor genes.

Glutathione S-transferase genes and cytochrome p450 genes are the main genes whose polymorphisms have been found to confer susceptibility to develop oral tumors (Hung 1997). There are numerous studies in India, which implicate polymorphisms in these genes and predisposition to oral cancer, especially in tobacco associated high-risk groups.

Glutathione S-transferase M1 and T1 enzymes are both known to catalyse detoxification of reactive oxygen species, lipid peroxidation products and tobacco-derived carcinogens.
that have been found in the saliva of Betel Quid (BQ)/tobacco chewers. Null genotypes of both \textit{GSTM1} and \textit{GSTT1} increase with high penetrance, separately or in combination, the risk for developing leukoplakia in an Indian ethnic population (Nair 1999). Polymorphisms in \textit{GSTP1, GSTM1, GSTM3} and \textit{GSTT1} genes regulate risk of cancer and leukoplakia differentially among different tobacco habituals (Sikdar 2004).

Increased relative risks of head and neck cancer were reported for the Alcohol dehydrogenase and Aldehyde dehydrogenase-\textit{ADH1B1/1} and \textit{ALDH21/2} genotypes in several studies. Individuals that carry the fast-metabolizing alcohol dehydrogenase type 3 \textit{(ADH3)} allele (Harty 1997) may be particularly vulnerable to the effects of chronic alcohol consumption and could be at increased risk to develop oral cancer.

\textbf{2.3.4 Human Papilloma virus (HPV)}

HPV has been detected in 31\% to 74\% of oral cancers and is also associated with papillomas, condyloma, verrucous leukoplakia, and carcinoma (Kashima 1990; Chang 1991; Vokes 1993; Franceschi 1996; Steinberg 1996). It is discussed in detail in chapter 4.

\textbf{2.3.5 Combination of risk factors}

The combined effect (tobacco and alcohol) is greater than the sum of the independent effects and probably multiplicative (Elwood 1984; Tuyns 1988; Franco 1989 ). The combination of smoking and HPV infection and of alcohol and HPV infection may have an additive effect.

\textbf{2.3.6 Diet}

A diet rich in fruits and vegetables, particularly fruit, reduces the risk of oral cancer and premalignant lesions (Mucci 2002). Several studies have shown that higher levels of vitamin C or carotene consumption reduce the risk of oral cancer. The potentially increased risk associated with meat consumption is less clear (Mehrotra 2006 ). Results of intervention studies involving dietary change or dietary supplements have shown no clear evidence of benefit (Mayne 2006). La Vecchia and his colleagues estimated that approximately 15\% of oral and pharyngeal cancer cases in Europe could be attributed to dietary deficiencies or imbalances (La Vecchia 1991).
2.4 Familial & Genetic predisposition

There is little evidence to suggest family history/genetic predisposition as a risk factor for oral cancer. Genetic predisposition has been suggested due to the fact that not all exposed to the risk factors develop oral cancer and sporadic cases of oral cancer occur in non-users of tobacco and alcohol and in young adults. Recently, many genetic events produced by chromosomal alterations caused by these risk factors have been proposed to underlie the histopathologic progression of oral squamous cell carcinoma (Califano 1996; El-Naggar 1996).

2.5 Dental factors

Wearing dentures *per se* is not a risk factor but chronic ulceration due to ill fitting dentures may promote a neoplasm in the presence of other risk factors (Lockhart 1998; Schildt 1998; Velly 1998).

2.6 Increased risk in immunocompromised individuals

Studies have reported that immunosuppressed patients (due to medications, bone-marrow transplants or disease) have an increased risk of oral cancer and premalignant lesions (Curtis 1997; Bhatia 2001; Quon 2004). One study reported a 11-fold increased risk of oral cancer for bone-marrow transplant patients (Curtis 1997). This risk increases with time, after transplantation.

2.7 Risk factors in Non-smoker Non drinker patients (NSND)

A descriptive study of the squamous cell carcinoma of the head and neck in never smoker–never drinkers has suggested that likely that no single known factor is responsible for a majority of SCCHN in NSNDs. Suggested factors are regular use of noncigarette tobacco products or marijuana, regular environmental exposure to tobacco smoke, regular occupational exposures to carcinogens/toxins, and history of gastroesophageal reflux disease and viral exposure (Dahlstrom 2008).

2.8 Risk factors in the young

Young patients (<45yrs) account for approximately 6% of all oral cancers (BritishDentalAssociation Occasional Paper No2000;6). Rising trends for oral cancer among young adults have been reported by studies in UK (Johnson 1993), Scotland (Macfarlane 1987; Macfarlane 1992; Macfarlane 1994), England, Wales (Hindle 1996) and in North Ireland (Cowan 1992), Europe (Levi 1995) and countries in Eastern Europe
like Slovakia (Plesko 1994). In USA, SEER data analysis has shown an increase in percentage of SCC tongue from 3% in 1973 to 6% in 1993 attributed to smokeless tobacco use (Davis 1987; Myers 2000). The incidence of oral cancers under 40 years is estimated to be between 16 and 28% of all oral cancers seen in various institutions in India (Patel 1976; Padmanabhan 1990). Kuriakose et al in India and Sarkaria and Harari in the USA reported young patients with cancers of the tongue having a low rate of tobacco use or betel–quid chewing relative to that observed in old patients (Kuriakose 1992; Sarkaria 1994).

It has been regarded that oral cavity cancers especially tongue cancers is predominantly a disease of the males (Prince 1999). This male dominance is not seen in younger patients probably due to the social acceptance of habits like smoking and drinking among women. Jones et al reported female patients with carcinoma of the tongue outnumbering males in patients under 40 years old (Jones 1989). Byers et al have reported similar findings (Byers 1975). The biological behavior of oral cancers in young may be different from that occurring in older patients.

2.9 Premalignant lesions- malignant potential

The premalignant lesions of concern are leukoplakia, erythroplakia, palatal lesion of reverse cigar smoking, oral lichen planus, oral submucous fibrosis, discoid lupus erythematosus, hereditary disorders such as dyskeratosis congenita and epidermolysis bullosa (Fig 2.3).

 Clinically recognizable premalignant lesions are particularly common in regions where the incidence of oral cancer is high. And 70% of oral cancers develop from premalignant lesions in high incidence countries. Many cancers of the oral cavity are preceded by clinically evident premalignant oral mucosal lesions, particularly erythroplakia and some leukoplakias. Except in restricted geographical regions, the disorders other than leukoplakia and erythroplakia contribute little to the burden of precancer or the incidence of oral squamous cell carcinoma. The vast majority of the lesions are associated with tobacco use, although 10% of lesions appear to have no known cause, the role of alcohol and HPV are unclear.
Leukoplakia

WHO defines leukoplakia as ‘a whitish patch or plaque that cannot be characterized clinically or pathologically as any other disease, and is not associated with any physical or chemical causative agent, except the use of tobacco.

In a systematic review (Petti 2003) based on 23 studies of oral leukoplakia from 17 countries published between 1986 and 2002, Petit calculated the global prevalence of 2.6% (95% CI 1.72-2.74%). Crude annual oral cancer incidence rate due to leukoplakia is likely to fall between 6.2 and 29.1 per 100000. The annual frequency of malignant transformation of all forms of leukoplakia is high ranging between 0-7% and 2.9% (Silverman 1984; Lind 1987; Schepman 1998). The fraction of oral cancer cases attributable to leukoplakia ranges between 17% and 35% (van der Waal 1997). The rates of malignant transformation ranges between 0.3% and 17.5% for leukoplakia (Reibel 2003).

The biomarkers that predict malignant transformation are degree of dysplasia, loss of heterozygosity of 3p14 and 9p21 region, genomic instability and DNA ploidy.

The following features are suggested to be associated with malignant transformation in oral leukoplakia (van der Waal 1997).
1. Females appear to be at higher risk.
2. Length of duration of the lesion.
3. Lack of obvious risk factors-idiopathic leukoplakia.
4. Location of the lesion- floor of the mouth, lateral border of tongue, ventral lingual mucosa appear to be high-risk sites.
5. Non-homogenous types.
6. Colonisation by fungi-Candid albicans.
7. Presence of epithelial dysplasia.

Leukoplakias related to smoking are more likely to persist than with chewing habits (Batsakis 2003). For smoking, previous studies showed that 70 to 90% of patients with leukoplakia were tobacco users and that 78% of leukoplakia lesions disappeared or regressed within 12 months after smoking cessation (Roed-Petersen 1971).

**Erythroplakia**

A term used to define oral mucosal lesions that present as red areas and cannot be diagnosed as any other definable lesion. It is a dangerous lesion with a higher rate of malignant transformation. Floor of the mouth is the most common site in men and alveolar mucosa, gingival and sulcus mucosa of the mandible are the common sites in women.

**Lichen planus**

It is a complex immunologically mediated mucocutaneous disease primarily common in adults especially women, Malignant transformation of lichen planus has been reported to be between 0.4% to 2.5% (Sigurgeirsson 1991).

**Submucous fibrosis**

It is an irreversible precancerous condition, which is strongly associated with the habit of chewing areca nut, very common in India. The patients experience burning sensation of the mucosa, occasional mucosal ulceration, peculiar marble like blanching of the mucosa and palpable fibrous bands of the buccal mucosa, soft palate and lips. The risk of malignant transformation is as high as 7% over a period of 15 years (Murti 1985).
2.10 Staging of oral cancer

Developed by the American Joint Committee on Cancer and International Union Against Cancer (UICC), the clinical staging is conventionally performed with the use of the “tumor, node, metastasis (TNM)” classification system and its variant (pTNM), which are respectively based on clinical and pathological assessment of tumor size and lymph node involvement.

2.11 Treatment

Various modes of treatment include surgery, radiotherapy, chemotherapy and combined modality treatments based on the site of the primary tumor, size, depth of infiltration, proximity to the bone, regional lymph node, and histology status.

Single modality treatment like either surgical resection or radiotherapy is preferred for early stage tumors (T1, T2). Patients with advanced stage of disease are candidates for combined modality treatment. Currently the role of chemotherapy is still investigational. Factors related to the patient like age, tolerance, general physical condition, occupation, socio-economic status, and factors related to the physician like surgical or radiotherapy skills, support and rehabilitation services influence the selection of initial treatment.

2.12 Prevention

Primary

Aims at elimination of risk factors from the community thereby minimizing the incidence. Physicians, dentists, health workers could play a major role in the elimination of risk habits. Also interventions can be made via schools, mass media, integrating health education with the existing community based programmes.

Secondary

Aims at detecting the disease at an early stage thereby reducing morbidity and mortality. Screening for oral cancer and precancers and chemoprevention come in this category. Oral cancer meets some of the criteria for disease screening. Oral visual screening is an effective tool because of the asymptomatic nature of the lesions, precedence by premalignant lesions and the detection of conditions like leukoplakia, erythroplakia and submucous fibrosis by simple visual examination. Population based screening cannot be recommended, but screening in high risk individuals is highly recommended (Sankaranarayanan 2005).
The over expressions of cyclooxygenase-2 (COX-2), phospho-epidermal growth factor receptor (pEGFR), activation of NFKB, RAR, and PCNA/Ki67 HPV gene expression and integration are important events in oral carcinogenesis and are the basis of targeted prevention strategies.

**Tertiary**

Aims at reducing the recurrence after treatment and reduction of morbidity after treatment.