INTRODUCTION

2.1 History of Cancer:

Ever since complex life evolved, it has been susceptible to cancer. The existence of cancer dates back to 70-80 million years as evident from cancer cells found in fossils of dinosaur. The oldest description of cancer in humans was found in an Egyptian papyrus written between 3000-1500 BC referring to breast tumor. In Greece in about 400 BC Hippocrates, the "Father of Medicine", is credited with being the first to recognize the difference between benign and malignant tumours. [http://info.cancerresearchuk.org]. Although, the oldest known possible hominid malignant tumor was found in Homo erectus or Australopithecus some 3.9-4.2 million years ago in 1932 making it no more a disease of modern industrialized age. The oldest specimen of a human cancer was found in the remains of a female skull dating back to the Bronze Age (1900-1600 BC). The mummified skeletal remains of Peruvian Incas, dating back 2400 years ago, contained lesions suggestive of malignant melanoma. Indian epic tales of Ramayana (500 BC) also describes treatment for thwart tumor growth with arsenic paste. Hippocrates was credited with being the first to recognize the difference between benign and malignant tumors. He called the disease "Karkinos" (in Greek means crab), as the swollen blood vessels around malignant tumors reminded him of crab claws and the term was translated to "carcinos or carcinoma" in English. Affecting millions of people every year, cancer is a disease of the cells.

Cancer or tumor is a term used for defining group of over 200 related diseases characterized by the disorderly and uncontrolled proliferation (clonal expansion) and spread of a single genetically transformed (abnormal) cell in the tissues of the body. It can arise in any site of the body and behave differently depending on its organ of origin. They are also classified according to the type of cell from which they originate into as carcinomas (epithelial cells of tissues and organs), leukemias (blood and blood forming organs), sarcomas (connective tissue, bone cartilage, fibrous tissue, muscle cells), and adenomas (glandular organs). These are further classified according to tissue or organ involved (e.g., breast or lung carcinomas). They can also be broadly classified as liquid or solid. The former includes leukemias and lymphomas,

composed of neoplastic cells whose precursors are normally mobile. While solid tumors are composed of epithelial or mesenchymal cells that normally are immobile.

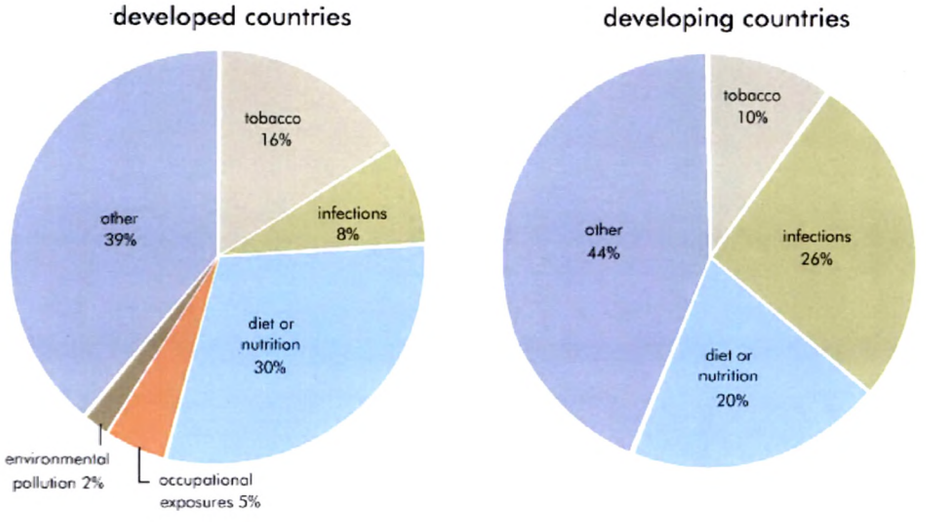
2.2 Variations in cancer incidence

There are striking variations in the risk, incidence as well as mortality of different cancers from person to person between different regions of the world owing to genetic make-up, ethnicity, ageing and environment factors. However, most of these variations of individual cancers largely attributes significantly to environmental component, as the genetic constitution of the mankind doesn't change and moreover they differs only moderately between different populations in different parts of the world. This environmental component includes human behaviour, habits and lifestyle that are potentially preventable. Once considered as a "western" disease, reports now highlights more than 50% of world's cancer burden already occurring in developing countries.

The main reasons for the greater cancer burden are the earlier onset of the tobacco epidemic, the earlier exposure to occupational carcinogens, western nutrition, low physical activity and lifestyle. However, with increasing wealth and industrialization, many countries undergo rapid lifestyle changes that will greatly increase their future disease burden. More than one million cases occur worldwide annually, with some 580,000 cases occurring in developed countries (>300/100,000 population per year) like U.S.A, Italy, Australia, Germany, Netherlands, Canada and France and the remainder in developing countries (usually<1500/100,000 population per year) like Northern Africa, Southern and Eastern Asia despite their much higher overall population and younger age. Over all, malignancies typical for developed countries are cancers of prostrate, breast, uterus, gallbladder, kidney, and colorectal cancers. While in developing countries, it shows that the incidence of cervical, oropharynx, esophagus and liver cancers is higher. It has been estimated that 43% of cancer deaths worldwide are due to tobacco, unhealthy diet, physical inactivity and infections [Stewart and Kleihues 2003]. Factors like tobacco

usage, infections, unhealthy diet or improper nutrition, occupational exposure, environmental pollution etc are the major risk factors for cancer incidence in both developing and developed countries (**Figure-2.2**). Early detection (screening) along with primary prevention by avoiding exposure to cancer-causing agents is the best strategy to control cancer and to improve survival of cancer patients.

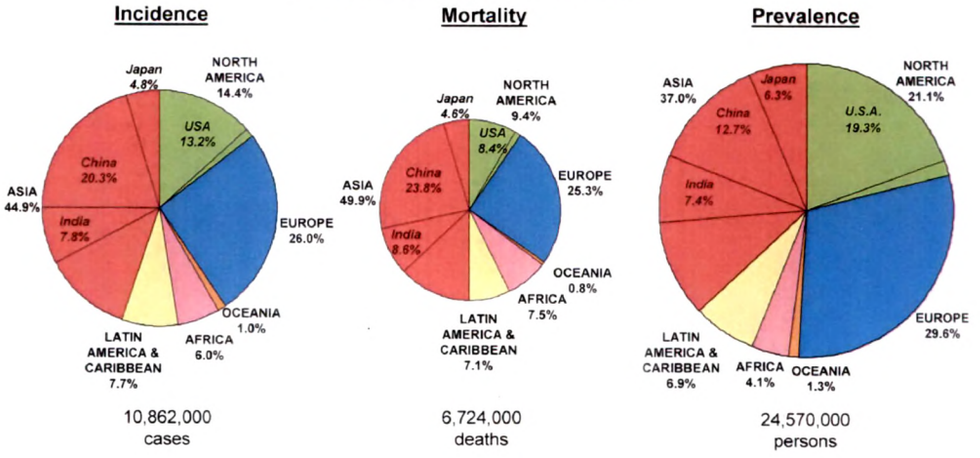
Figure-2.2: Major risk factors attributing to cause of cancer.



[Adapted from: The Cancer Atlas, 2006; http://www.cdc.gov]

2.3 Global cancer burden: Cancer is a devastating disease whose global burden continues to rise in 21st century as a major threat to public health affecting mankind. It continues to rank among the world's deadliest and most costly diseases. It stands as one of the top 15 leading causes of death affecting mankind in 2005. Among them cancers of lung, trachea, bronchus, stomach, breast, colon and rectum top the list. [WHO report, Fact sheet N° 310 / February-March 2007]. According to the World Health Report (WHO, 2004), cancer accounted for 7.1 million deaths in 2003 and it is estimated that overall number of new cases will rise by 50% in the next 20 years [WHO, 2003].

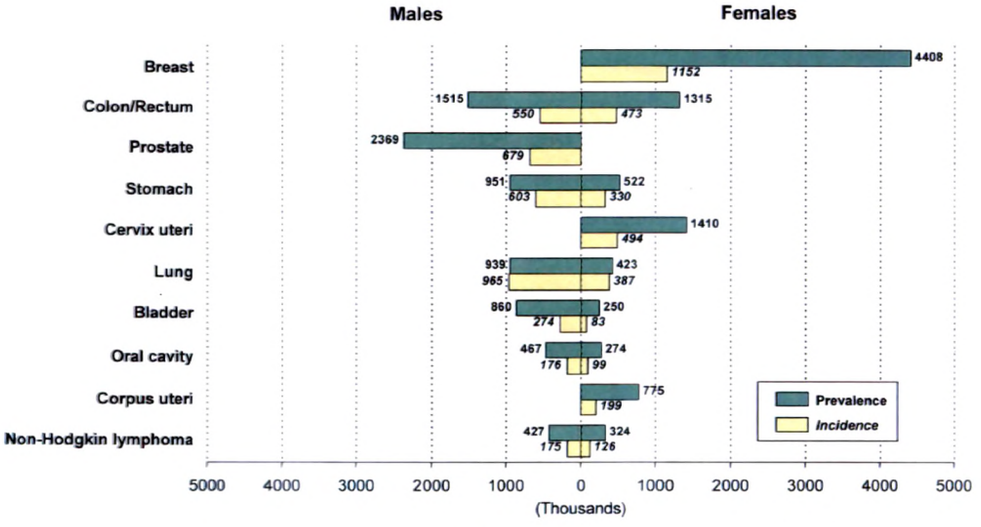
Figure-2.3.1: Incidence, mortality and prevalence of cancer worldwide by continent.



[Reference: Parkin et al, 2005, Global Cancer Statistics, 2002].

It is believed that one out of three persons will be treated and succumb to severe cancer in their lifetime. Infact by 2020, more than 16 million new cancer cases and 10 million deaths are expected. Parkin et al have reported global cancer burden addressing worldwide incidence, mortality and prevalence of 26 major types of cancer [Parkin et al, 2005]. The study estimated that in 2002, there were 10.9 million new cases, 6.7 million deaths, and 24.6 million persons alive with cancer (within 5 years of diagnosis). China and North America were rated as first and second in the list accounting 2.2 million (20.3 %) and 1.6 million (14.4%) of the world's total new cancer cases. Asia, Europe and North America show the highest incidence, mortality and prevalence of new cancer cases among different continents in the year 2002 (**Figure-2.3.1**).The most commonly diagnosed cancers are lung (1.35 million), breast (1.15 million), and colorectal (1 million); the most common causes of cancer death are lung cancer (1.18 million), stomach cancer (700,000), and liver cancer (598,000). The most prevalent cancer in the world is breast cancer (4.4 million survivors up to 5 years following diagnosis). In males, the top major cancers reported were lung, prostrate, stomach, colorectal, bladder, oral cavity and lymphoma, while in females, cancers of breast, cervix-uteri, colorectal, lung, stomach, ovary, lymphoma, oral cavity and bladder listed the top (**Figure-2.3.2**).

Figure-2.3.2: New cancer cases (incidence) and prevalent cases (five year survival) in thousands by cancer site in 2002.



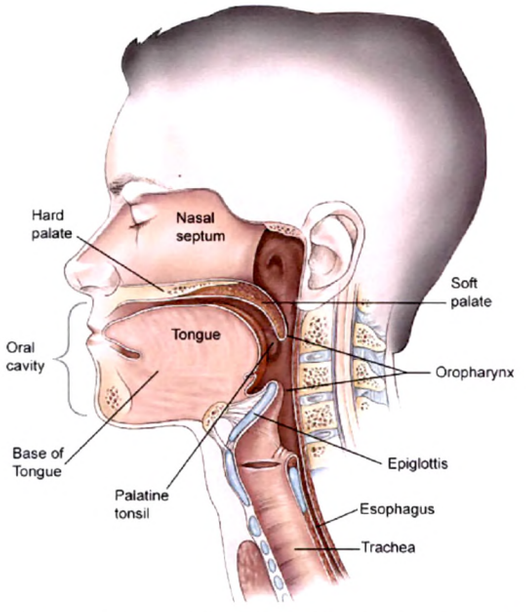
[Reference: Parkin et al, 2005, Global Cancer Statistics, 2002].

Oral cancer scenario worldwide and in India

Cancers of head and neck are the sixth most common cancers in the world with oral cancer representing a great public health problem. Most oral cavity cancers are squamous cell carcinomas (SCC) and the subsites include: lips (excluding skin of the lip), tongue, buccal mucosa, floor of mouth, hard palate, upper and lower gingivae, mouth, salivary glands, pharynx, oropharynx, and hypopharynx (**Figure-2.3.3**). Oral cancer is a significant disease globally with an estimated 390,000 new cases worldwide accounting for 2% to 3% of all malignancies [Parkin et al, 2002; Silverman et al, 2001; Llewellyn et al, 2001; Swango, 1996; Chen et al, 1990; Silverman et al, 1990]. It is also one of the 10th most frequently occurring cancers diagnosed annually with 2/3rd of these cases occurring in developing countries [Stephen et al, 2004; Parkin et al, 1999] with rising trends observed in young and middle-aged men [Llewellyn et al, 2001]. The incidence of oral cancer significantly varies among different countries and regions, from approximately 1 per 100,000 males in Egypt and China and to around 5 in the UK and Argentina, to almost 10 in USA, close to 15 in India, Australia, and Croatia, 20

in Hungary and 25 in Srilanka [Rumboldt et al, 2006; Stewart and Kleihues 2003, Peterson 2003; IARC. GLOBOCAN 2002, 2004].

Figure-2.3.3: Head and neck sites in human body



Adapted from: American Society of Clinical Oncology, 2003

Recent data from the Surveillance, Epidemiology, and End Results (SEER) Program suggest that 28,900 new cases of oral cancer have been identified and 7400 deaths attributed to oral cancer each year in the United States in 2002 [Jemal et al, 2004; Ries et al, 2005; Mao et al, 2004]. The incidence and mortality from oral cancer is rising in several regions of the world, including Europe, South Central Asia (particularly Taiwan and Japan) and to a lesser extent in Australia, Japan, New Zealand and USA [Stewart and Kleihues 2003, Peterson 2003, Parkin, 2002]. In some regions, the prevalence of oral cancer is much higher, representing approximately 10% of all cancers in Pakistan, and around 45% in the Indian subcontinent as a whole due to etiological differences [Siddiqui, 2000]. Though the incidence has decreased, it still has a significant mortality with 128,000 deaths recorded representing nearly half of the incident cases (48%) [Parkin, 2000]. The five-year relative survival rate is estimated at 59.1% overall for oral cancer diagnosed during 1996–2003

[Denis, 2007]. Unfortunately, overall, neither incidence nor mortality of cancer has been much diminished by conscious human intervention.

2.4 Oral cancer scenario in India

Global comparison shows that India has the highest incidence rates of oral cancer in the world and seems to be still rising making it a major health problem [Peterson, 2005; Fenley et al, 2001; Davidson, 2001; Shah et al, 2001; Hamada, 1991; **www.whoindia.org.**]. In parts of Southeast Asia (particularly India), oral squamous cell carcinoma(OSCC) accounts for 40% of all malignant diseases [Johnson 2001; Moore et al, 2000; Parkin 1998; Parkin, 1980; Pindborg , 1971]. Annual estimates of incidence of cancer in India is 0.98 million and mortality is 0.7 million (ICMR, 2001). 179 million persons were diagnosed with cancer in India from the year 1998-2002. In India there are 75,000-80,000 new cases of oral cancer registered each year [ICMR, 2004; National cancer Registry Programme, 1996]. The age standardized incidence rates for oral cancer per 100,000 was 12.8 and 7.5 in men and women per year respectively [Ferlay et al., 2001]. It constitutes 12% of all cancers in men and 8% of all cancers among women making it the highest in the world due to higher tobacco consumption and exposure [Nair et al, 2004; Rani et al, 2003; Sankanarayanan 1990].

2.5 Incidence of oral cancer at The Gujarat Cancer and Research Institute: Oral cancers have been one of the most common cancers in regions of Western India especially in Ahmedabad [Davidson, 2001; Shah et al, 2001; Sanghavi et al., 1989]. An increasing trend has been observed in a number of oral cancers cases registered in the hospital cancer registry data of The Gujarat Cancer and Research Institute (G.C.R.I), Ahmedabad during the consecutive years from 1996 to 2003 [Hospital based cancer registry, 2003]. The top 15 cancers registered at G.C.R.I. are shown in the **Figure-2.5.1** where cancers of cervix-uteri, breast, esophagus, base of tongue and other parts of mouth unspecified were the highest irrespective of sex. In the year 2003, the number of cancer cases reported were 11, 851 among which the

male to female ratio for all cancers was 1.46:1. While lung (10.42%) and base of tongue (7.6%) were the highest among males and cervix-uteri (24.19%) and breast cancers (21.09%) were the highest among females.

Figure-2.5.1: Top major cancers by site irrespective of gender registered at G.C.R.I., 2003.

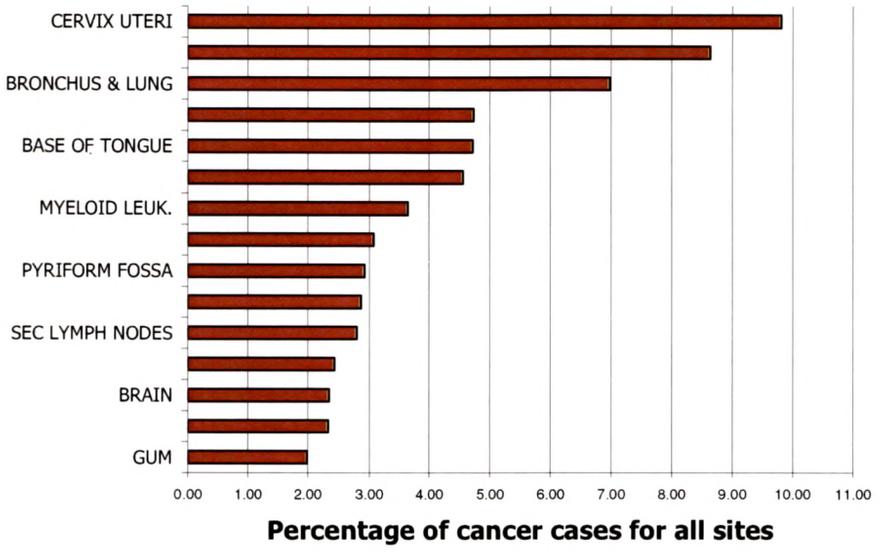
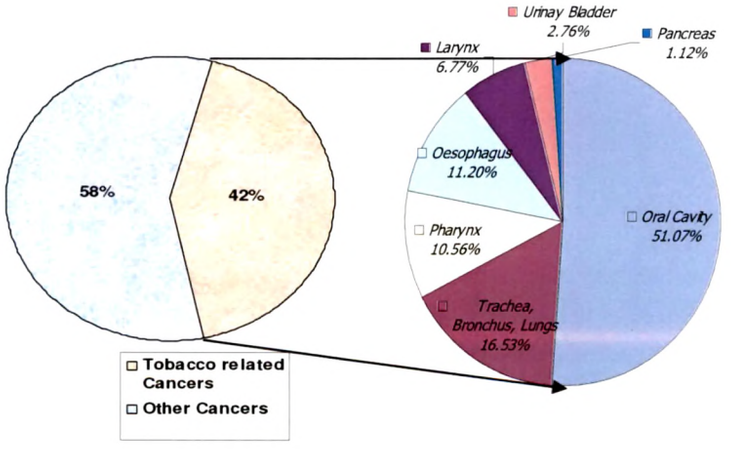


Figure-2.5.2: Incidence of Tobacco related cancers at G.C.R.I., 2003.



Among all cancers, tobacco related cancers (TRC) comprised of 5009 number of cancer cases accounting 42.3%. Of these, the cancer of oral cavity (International Classification of Diseases; ICD10: C00-09) was the leading site

accounting 51.07% of TRC cases and 21.6% of all cancers registered at G.C.R.I. as pictorized in the pie chart below (**Figure-2.5.2**). The ratio of oral cancers observed in males and females was 3.09:1. **Table-2.5.1** shows the most common sites for oral cancer listing cancers of tongue, pyriform fossa and gingivae (gum) at the top.

2.6 Etiology and risk factors for oral cancer

No single factor can be considered to be the sole causative for oral cancer development. The complex process of oral carcinogenesis involves dynamic interactions among many factors. These factors may include gene-environment interactions, host factors like genetics, immune function, hormonal factors, environment like contaminants and occupational exposure and lifestyle factors like tobacco, alcohol, diet etc (**Table-2.6.1**) [Travis, 2002]. Striking variations in oral cancer sites and incidence are seen among different regions, cultures, and demographic groups, due in large part to differing patterns of tobacco and other substance abuse.

Table-2.5.1: Oral cancer incidence in G.C.R.I. by site [Hospital based cancer registry, 2003].

Site of cancer	Percentage of cases
Tongue	36.04%
Pyriform fossa	13.53%
Gum	9.19%
Tonsil	8.91%
Floor of mouth	2.19%
Lip	2.15%
Parotid gland	1.56%
Minor salivary glands	0.74%
Other parts of oral cavity	25.68%

Age: It is frequently a risk factor for oral cancer, as historically oral cancer was typically associated with men aged 60 years and older [Reibel 2003; Johnson 2001; Lewin et al, 1998]. A steady increase in the incidence of oral

cancer (range 0.4%–4.0%) has been observed in patients younger than 40 years where confounding factors include genetic susceptibility and immunologic profile regardless of social class or sex irrespective of gender are particularly affected [Rumboldt et al, 2006; Schantz and Yu, 2002; Llewellyn et al, 2001; Singh et al, 1998].

Gender: Although, in the past the prevalence of oral cancer was particularly high in men, but the rate also seems to be dramatically increased in women narrowing the gender gap [Cancer Research Campaign-Oral cancer, Fact sheet 14:1-14.5, 1993]. The male to female ratio has changed from 10.1 to 2.1 or 3.1 [Kademani, 2007]. Incidence rates for oral cancer vary in men from 1 to 10 cases per 100,000 population in many countries. Incident rates of oral cancer were found to be higher in men in Western Europe (11.3 per 100,000), Southern Europe (9.2 per 100,000), South Asia (12.7 per 100,000), Southern Africa (11.1 per 100,000), and Australia/New Zealand (10.2 per 100,000). In females, incidence is relatively high in Southern Asia (8.3 per 100,000).

Race: Race has also been considered as risk factor for oral cancer. Higher risk is seen in black as compared to whites. Moreover, black people have markedly lower survival rate i.e. 35%. In essence, 33% higher incidence and 100% higher mortality are seen in black than in white people [Kademani, 2007).

Viruses: The incidence of HPV-related oral cancer is relatively low making its association unclear, but more recent reports have strengthened its etiological role as a risk factor [Eric et al, 2001]. High risk subtypes of HPV (Human papilloma virus) 16 and 18 were found to be associated with 22% and 14% of oropharyngeal cancers respectively and they increase the risk of oral cancer by 3 to 5 fold independent of smoking or drinking habits [Denis, 2007; Gillison and Shah, 2001; D' Souza et al, 2007, Rosendquist, 2005]. While low risk HPV subtypes –6 and –11 are more commonly found in oral cancer development. These viruses have known to be potentially transmitted to oral mucosa via sexual route; and believed to contribute to oral carcinogenesis by modulating

expression of some tumor suppressor genes. But the prognostic implications of its presence and integration are still to be determined.

Diet: There has been found to be a protective effect of consumption of carotene rich vegetables and citrus fruits [La Vecchia 1997; Macfarlane, 1995]. Low intake of dietary fibers, deficiency of vitamins like Vitamin A, B12, C, E, folic acid and minerals like iron may be associated with risk of oral cancer, as they might cause impaired synthesis, repair and methylation of DNA [Denis, 2007; Pavia et al, 2007]. They also aid in scavenging potentially mutagenic free radicals from damaged cells.

Other contributing risk factors: There is also strong evidence that low socio-economic status is associated with a higher incidence and poorer survival of oral cancer [Faggiano, 1997]. Additional contributing risk factors are those associated with exposure to a variety of biological, chemical and physical agents such as infection with Treponema pallidum, Herpex simplex virus, or C. albicans; oral neglect; chronic trauma; ultraviolet radiation (for lip cancer) and HIV seropositivity, use of areca nut, narcotics and genetic factors.

Tobacco as radical cause of oral cancer

For oral cancer, at least 75% of those diagnosed are tobacco users. Tobacco is thought to originate either from a Spanish word "tabaco" in Taino language of the Caribbean, referring to a roll of these leaves (according to Bartolome de Las Casas, 1552) or from "tabago", a kind of y-shaped pipe for sniffing tobacco smoke (according to Oviedo, the leaves themselves were referred to as Cohiba, but specifically tobacco was commonly used to define medicinal herbs from 1410, originating from the Arabic word "tabbaq", reportedly since the 9th century. It is an agricultural product processed from the fresh leaves of plants in the genus Nicotiana grown in American Continent since about 6000 BC but began to be used in one form or another since about 2000 BC. In 1609, tobacco was often referred as a "brown gold" successfully rose for commercial use at Jamestown [http://en.wikipedia.org/wiki/Tobacco].

Approximately 100 million people died worldwide from tobacco - associated diseases.

Table-2.6.1: Established risk factors for the development of oral cancer

Predisposing risk factors	Increasing age Male gender Genetics Socioeconomic status
Risk factors	Smoking, chewing tobacco /oral snuff Heavy consumption of alcohol Betel quid chewing Areca nut use HPV positivity HIV seropositivity Previous oral lesion or other cancer
Contributing factors	Use (abuse) of narcotics Cannabis use Sunlight exposure (lower lip) Candida albicans infection Dental trauma or chronic sepsis Exposure to burning fossils fuels Anaemia Immune deficiency or immune suppression Familial or genetic predisposition
Negative association	High dietary fruit Vegetable intake Adequate intake of Vitamin A, C, E, Folate and Iron

It is believed that half of all regular tobacco users are killed by the habit and one quarter die prematurely before the age of 70. Tobacco use is responsible for the death of one in ten adults worldwide as a major cause of many of the world's top killer diseases–including cardiovascular disease, chronic obstructive lung disease and lung cancer. Approximately 80% of patients have history of tobacco usage in any form is projected to kill 50% more people in 2015 than HIV/AIDS, and to be responsible for 10% of all deaths globally. In 1999, Gupta propagated oral cancer in India as a "new epidemic" due to its higher incidence attributed to wide spread habits of tobacco use in varied forms as compared to western countries [Gupta 1999]. It is considered

as a sole most independent potential avoidable risk factor responsible for oral carcinogenesis. Tobacco is largely consumed by varied genre of Indian population [Gupta and Nandkumar, 1999]. Its association as an etiological factor for oral squamous cell carcinoma (OSCC) development and field cancerization has been confirmed by clinical, laboratory and epidemiological studies [Nair, 2004; Gupta, 1987]. The evidence that smokeless tobacco causes oral cancer was confirmed recently by the International Agency for Research on Cancer [Cogliano, 2004]. In India it is estimated that among the 400 million individuals aged 15 years and over 47% use tobacco in one form or the other [Mehta et al, 1993]. Tobacco use is a well-established cause of OSCC and field cancerization [Slaughter, 1953]. Approximately 80% of patients with OSCC have smoked or used tobacco products and the incidence is directly dependent upon the amount and duration of the habit; these patients have 5 to 7 times greater risk of developing malignant head and neck tumors than nonsmokers [Lewin, 1998; Brennan, 1995; Andre, 1995; Mashberg, 1993]. High prevalence of oral cancer in India was primarily because of the most common form of tobacco consumption is keeping the tobacco in mouth. Be it in the form of Gutka, Quid, snuff or misri and so on. They have 5-7 times higher risk of developing malignancy than non-smokers. Reverse smoking revealed that use of tobacco in this form conferred a 5.19 times higher risk than did use of chewing tobacco [Hebert 2002]. The oral cancer risk increases when tobacco is used in combination with alcohol or areca nut [Reibel, 2003]. The number of cases reported for oral cancer for both sexes has taken toil due to over consumption of tobacco in smoke and majority using in smokeless form.

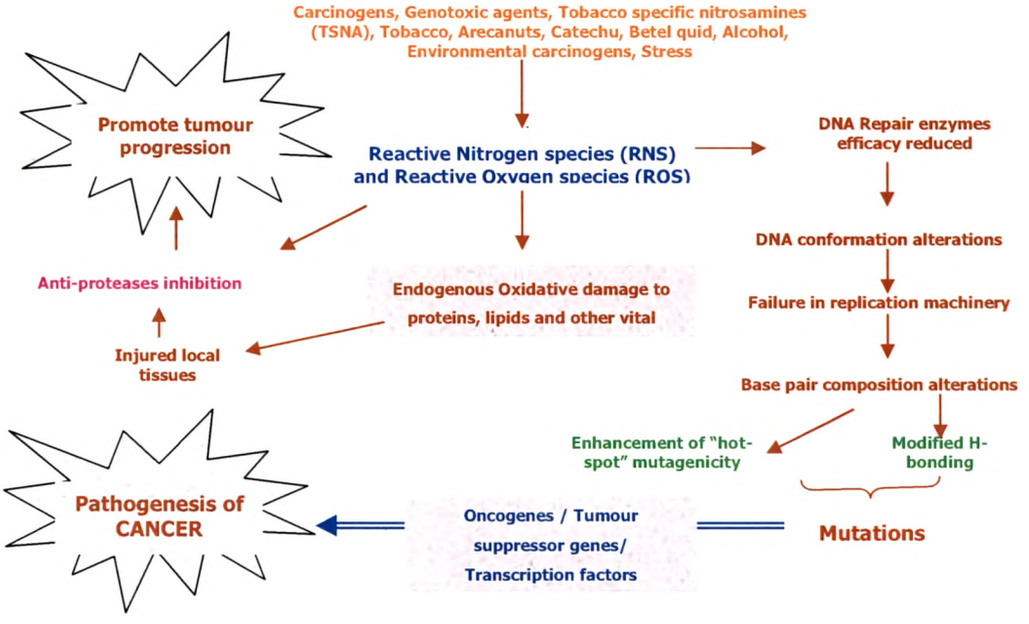
Common forms of tobacco consumption: In India, tobacco is used in varied forms- smoke and smokeless forms. Tobacco is smoked either in form of chillum, cigarettes, cigars and pipes, dhumti, chillum, hookah, cheroots or reverse smoking (i.e. placing the lit end of the cigarette into the mouth). But high prevalence of oral cancer is mainly attributed to use of tobacco in the smokeless forms like chewing {gutka, betel quid (mixture of slaked lime catechu, betel leaf and tobacco), arecanuts, pan masala, mava} or, snuffing

(dipping/ inhaling tobacco burnt powder after processing) as shown in **Figure-4 (prologue section)**. The slaked lime releases an alkaloid from the areca nut, causing a feeling of euphoria. Chronic use may lead to a debilitating trismus from submucal fibrosis, which has a high rate of malignant transformation [Murti et al, 1995; Pindorg et al, 1984]. The tobacco when kept in mouth leaches out carcinogens, which induce free radical oxidative damage on the mucosa causing precancerous lesions, which lead to cancer. Patients who continue to smoke after diagnosis of an initial tumor have up to a 6-fold greater risk of developing a second primary tumor in the aerodigestive tract than patients who stop smoking [Johnson, 2001; Blot, 1988; Silverman, 1972]. Use of smokeless tobacco tends to incite malignant degeneration only at the site of tobacco placement (not a generalized field effect) [Grady, 1998; Bouquot, 1998], and is associated with development of OSCC, albeit at a considerably lower rate than that associated with traditional tobacco use [Lewin, 1998; Kaugars, 1992; Winn, 1981].

Carcinogens in tobacco: Habitual use of tobacco in the form of chewing and smoking are equally dangerous. It initiates a linear dose-response carcinogenic effect in which duration is more important than the intensity of exposure. Tobacco either smoked or when kept in mouth with other combinations has a high carcinogenic potential, leaching out carcinogen that act on oral mucosa causing neoplastic changes. It is known to contain more than 100 potent carcinogens including nicotine and cotinine, alkaloids, nornicotine, anabasine, anatabine, arecoline, tobacco specific nitrosamines, nitrosodiethanolamine, nitrosoproline, polycyclic aromatic hydrocarbons (PAHs) and polonium. Smoke of tobacco contains nickel, cadmium, carbon monoxide, thiocyanate, hydrogen cyanide, phenol, volatile aldehydes, benzopyrene, nicotine and metabolites of these constituents. Tobacco specific nitrosamines (TSNA) include – 4 (methylnitrosamino) -1 (3-Pyridyl) – 1 butanone NNK, N – nitrosonornicotine (NNN), 4 – Methyl nitrosamino – 1 (3 pyridyl) – 1 – butanol (NNAL) [Hecht and Hoffman, 1988; 1983]. The major carcinogenic activity of smoke resides in the particulate (tar) fraction, which

contains a complex mixture of interacting cancer initiators, promoters, and co-carcinogens. The association between tobacco derived co-carcinogens and oral cancer has been very well studied by various groups [Nair et al, 2004, 1995]. It contributes to oral carcinogenesis in a multistep process by continuous generation of important class of genotoxic carcinogens like TSNA, reactive nitrogen species (RNS) and reactive oxygen species (ROS) that cause formation of DNA adducts through endogenous oxidative damage [Nair et al, 1987]. **Figure-2.6.1** shows how these carcinogens and genotoxic agents induce free radicals damage during processing of tobacco in saliva and causing enough damage to vital biomolecules of body implicated in pathogenesis of cancer [Sultan et al, 2004; Ray et al, 2002; Nair et al, 1995].

Figure-2.6.1: Implications of ROS and RNS mediated damage in pathogenesis of oral cancer



These reactive species are extremely unstable and molest the nucleophilic centers of cellular DNA causing formation of DNA adducts due to which the genetic material of the cells of oral cavity is sacrificed leading to accumulated

mutations in critical machinery of genes affecting proteins and their expression finally modulating the biological function [Loechler et al, 1984].

Chronic Alcoholism: Although primary, tobacco is not the only factor in the complex causality equation for oral cancers. Alcohol is also one of the most potential carcinogens owing to its multiple mutagenic effects on DNA, contributing to 3.6% of all cancers worldwide [Baan et al, 2007]. Moreover, countless epidemiological data from cohort and case-control studies proposed alcohol as a major risk factor for various cancers [Boffetta and Hashibe, 2007; Boffetta, 2006]. Up to 50% of patients diagnosed with oral cancer have a clinically significant history of alcohol use [Elwood, 1984]. If consumed along with tobacco, it works synergistically to increase mucosal permeability to carcinogenic nitrosamines that increases the risk of malignant degeneration by more than 100 times in case of heavy smokers and drinkers [Ries, 2006; Lewin, 1998; Blot, 1988]. It potentiates the carcinogenic effect of tobacco at every level of tobacco use, and the causative effect is most striking at the highest levels of exposure to both. Between 34% and 57% of patients continue alcohol and tobacco consumption even after diagnosis of oral cancer; this behavior increases the risks and a complication associated with surgery, increase the likelihood of recurrent cancer, and reduces disease-specific survival [Miller, 2004; Day, 1994].

2.7 Symptoms of oral cancer: The average duration of symptoms is usually around 4-5 months, ranging from a few weeks up to one year. Some patients may be asymptomatic and have occult oral lesions that are detected during routine screening while, symptomatic patients may present with nonspecific pain, mucosal ulcer with localized mass, often referred as pain in the mouth, teeth, throat, ear etc. Some patients may also present with symptoms like swelling or lumps on lips, gums or any other area of mouth, soreness on face and neck, thickening of oral soft tissues, white or red patches in the mouth, loose teeth, persistent sinusitis, nasal obstruction, bleeding, bad breath, dysrathria (difficult in speech articulation), dysphagia (difficulty in swallowing), odynophagia (pain while swallowing), otalgia (ear

pain), numbness in any area inside mouth, sensory and motor nerve compromise, mass lesions at the primary site or cervical lymphadenopathy. Majority of the oral cancers are diagnosed when they become symptomatic. At least 50% decrease in survival rate has been attributed to late diagnosis.

2.8 Staging of oral cancer: Most important factor in determining the appropriate treatment, survival and prognosis of cancer is the staging of the disease at the time of presentation. Staging is a way of describing cancer, such as where it is located, if or where it has spread, and if it is affecting the functions of other organs in the body. It not only helps in assessing the extent of local, regional, and distant disease but also provides information about the functional and performance status of patients, which further aids in guiding therapy. Oral cancer patients are currently staged and assessed using a combination of tumor size or depth (T), lymph nodes spread (N), and presence and absence of metastasis (M) [TNM] classification system promulgated by American Joint Committee on Cancer (AJCC) Staging Criteria incepted in 1958 (**Table-2.8.1**). The TNM classification is basically a clinical description of the disease exclusively on the anatomical extent of disease, but can also involve imaging in classification.

One of the illustrations where staging of head and neck cancers is depicted is shown in **Figure-2.8.1**. Oral cancers are also given histopathologic grades, which correlate with differentiation of the tissue and the clinical aggressiveness of the lesion. It describes how closely the cancer cells resemble normal tissue. Normal tissue contains many different types of cells grouped together, which is called differentiated. Tissue from tumors usually has cells that look more alike each other (called poorly differentiated). Generally, the more differentiated the tissue, the better is the prognosis. A tumor's grade is described using the letter "G" and a number. GX: The grade cannot be assessed. G1: The cells look more like normal tissue (well differentiated). G2: The cells are only moderately differentiated. G3: The cells don't resemble normal tissue (poorly differentiated).

Table-2.8.1: Staging of head and neck cancer

Staging of head and neck cancer according to American Joint Committee on Cancer (AJCC)			
Classification - TNM System			
Stage	**T**	**N**	**M**
0	Tis	N0	
I	T1	N0	M0
II	T2	N0	M0
	T3	N1	M0
III	T1	N1	M0
	T2	N1	M0
	T3	N0	M0
	T3	N1	M0
IV	T4	N1	M1
	T4	N2*	M0
	T4	N3	M1

T –Tumor size or depth
N – Lymph node involvement
M –Metastasis

Tumor size
Tis-carcinoma insitu
T0- No evidence of primary tumor
T1- Primary tumor size <2cm
T1b - >5cm but not more than 1cm in greatest dimension.
T2- Primary tumor size 2cm-5cm
T3- Primary tumor size >5cm
T4 –of any size but with direct extension to adjacent structures (e.g. muscle, chest wall, skin, bone, nerves etc.)

Nodal status
N0 -No regional lymph nodes metastasis
N1 - Metastasis to a single ipsilateral lymph node (<3cm) in greatest dimension
N2a: Metastasis to a single ipsilateral lymph node (3-6 cm)
N2b: Metastasis to a multiple ipsilateral lymph nodes (<6 cm)
N2c: Metastasis to bilateral/contra-lateral lymph nodes (<6 cm)
N3: Metastasis to any lymph node (<6 cm)
N2*=N2a/b/c

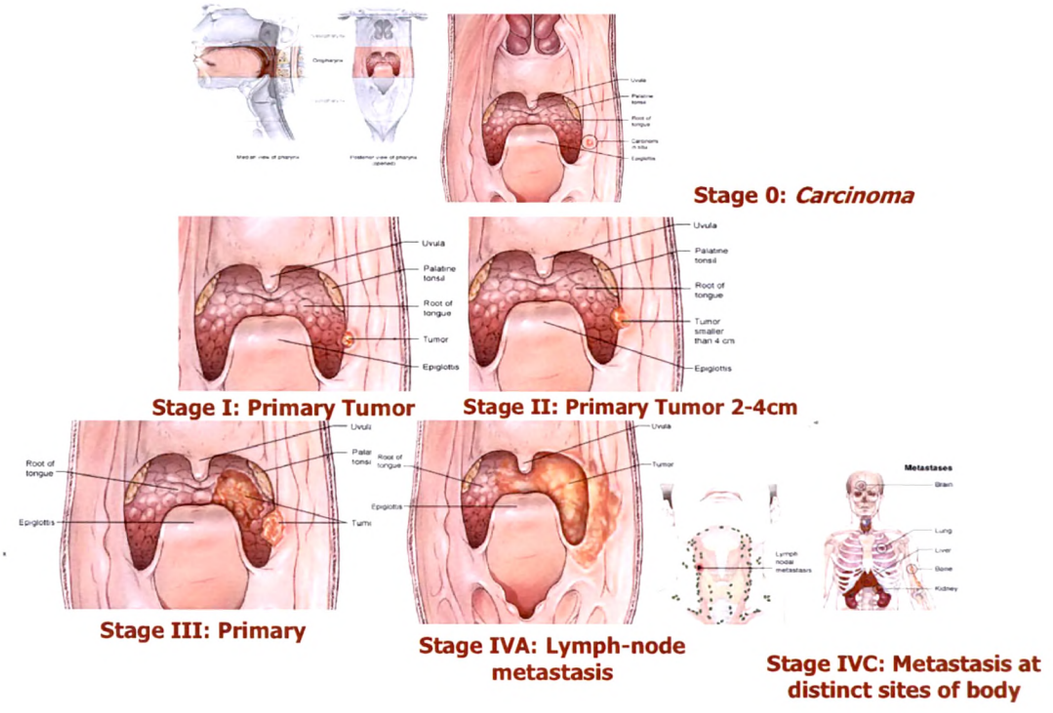
Distant metastasis
Mx- cannot be assessed
M0-No distant metastasis
M1- Distant metastasis

[Reference: American Joint Committee on Cancer (AJCC), 1997]

2.10 Survival of oral cancer

Oral cancer is associated with significant morbidity and mortality. Early diagnosis can efficiently reduce morbidity and minimize the extent of treatment required. The prognosis of cancer and so the survival rate varies from better to worse as the stage of cancer progresses from localized to metastasis (Table-2.10.1). It is clear that early diagnosis of oral cancers significantly improves the patient's long-term survival. Survival rates for early-stage disease are around 80%, whereas patients with advanced-stage disease (stages 3 and 4) have survival rates of 21% [Ries, 2006]. The overall five-year relative survival rate for oral and pharyngeal cancer is estimated at 59.1% for cases diagnosed during 1996–2003, which is increased slightly from the 1970s when the five-year relative survival rate was approximately 53% [National Cancer Institute. SEER Cancer Statistics Review 1975–2004. Available at: seer.cancer.gov, 2007].

Figure 2.8.1: Pictorial illustration of different stages of cancer.



Adapted from American society of Clinical Oncology, 2003

The best prognosis exists for lip cancer, with a 97% five-year relative survival rate if the tumor is localized and completely excised. Patients with oropharyngeal cancers with distant metastasis are estimated to have only a 26.5% relative survival rate. Survival rates also depend on the cancer's site (**Table-2.10.2**). Lip cancer usually presents as a lesion that fails to heal and is obvious to the patient and clinician alike, and has a high survival rate if diagnosed early and treated appropriately. Fifty percent of tongue carcinomas have metastasized by the time they are diagnosed, and within five years a further 20% metastasize [Landis, 1998].

Recurrence: Local tumour recurrence and the development of multiple primary tumours are the major causes of treatment failure, which occur in 10-30% of the cases with advanced tumors, even with histologically tumor-free surgical margins [Leemans et al, 1994; Partridge et al, 2000; Mayers et al, 2001; Shah 2001; Woolgar 2003]. 4.3% to 30% of oral cancer patients subsequently develop second primary tumours (SPTs) of the upper aero-

digestive tract [Day, 1992; Hong et al, 1990] attributed to alcohol and tobacco consumption as a result of field cancerization. Although loco-regional control has improved during the last decades, 5-year mortality rate of ~50% [Rafael et al, 2006] which has not changed significantly in >50 years have not changed accordingly, which can be explained by a higher frequency of distant metastasis occurring most commonly in lungs, bone and retroperitoneal lymph node [Leemans et al, 1993].

Table-2.10.1: Five-year relative survival rate by site and stage of disease

Stage	Five-year survival rate for oral cancer
Localized	81.80%
Regional	52.10%
Distant	26.50%
Unstaged	46.2%
Overall	59.10%
Adapted from: SEER Cancer Statistics Review 1975-2004	

Table-2.10.2: Five-year relative survival rate for different sites of oral cancer in percentage by stage of disease

Site of Cancer	Overall	Localized	Regio-nal	Distant	Unsta-ged
Tongue	54.30	73.6	46.80	25.90	42.30
Floor of mouth	59.70	80.90	47.7	27.20	54.10
Salivary gland	51	65.30	53.3	28.0	38.50
Lip	93.80	97.0	80.2	35.30	89.0
Gum, other mouth	73.90	94.20	58.60	32.70	56.60
Other oral cavity, Pharynx	29.90	49.20	29.50	4.70	32.50
Other Oro-, Nasopharyngeal	29.2–52.8	49.20-78.5	30.4-57.6	12.9-30.6	28.8-51.7
Adapted from: SEER Cancer Statistics Review 1975-2004					

Moreover, the presence of lymph node metastasis is the most important prognostic factor in head and neck carcinoma observed in 15 to 25% of the patients. Survival may decrease by ~50% and the possibility of distant metastasis may increase when there is cervical node involvement [Sang-chul et al, 2004; Leemans et al, 1994].

2.11 Treatment of oral cancer

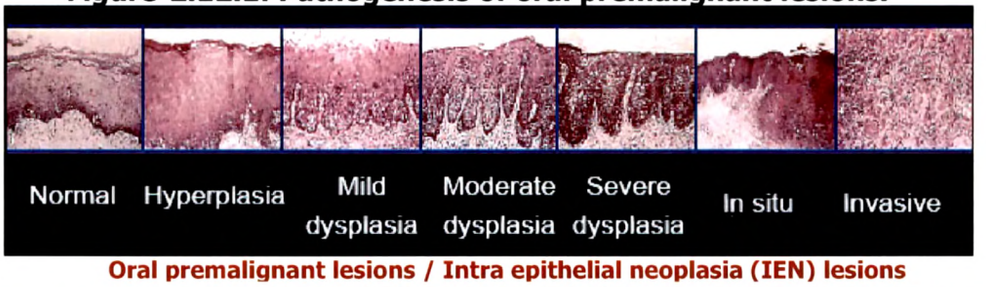
The prognosis for oral cancer depends, in large measure, on the site involved (posterior tumors having worse prognosis), the clinical stage at the time of diagnosis and treatment, tumor diameter, the patient's access to adequate health care, and their ability to cope and mount an immunological response. Because early treatment is paramount, biopsy should be done if neoplasia is suspected. Treatment of oral cancer can be done by surgery, radiotherapy, chemotherapy or combinations of these. The optimal treatment modality (surgery alone, radiotherapy alone, chemotherapy alone or some form of combinational therapy) for oral cancer is still controversial [Haddadin et al, 1999]. In general, stage I and stage II cancers require one modality of treatment, either surgery or radiation therapy to successfully control the cancer [Day et al, 2003]. Advanced stage III and stage IV cancers will often require combinations of surgery, radiation therapy and chemotherapy or even the use of all three. The management of advanced oral cancers is problematic and has traditionally relied on conventional forms of therapy; however the prognosis remains poor for these patients, where 70% of cases after treatment undergo relapse finally succumbing to death.

2.12 Pathogenesis of oral cancer

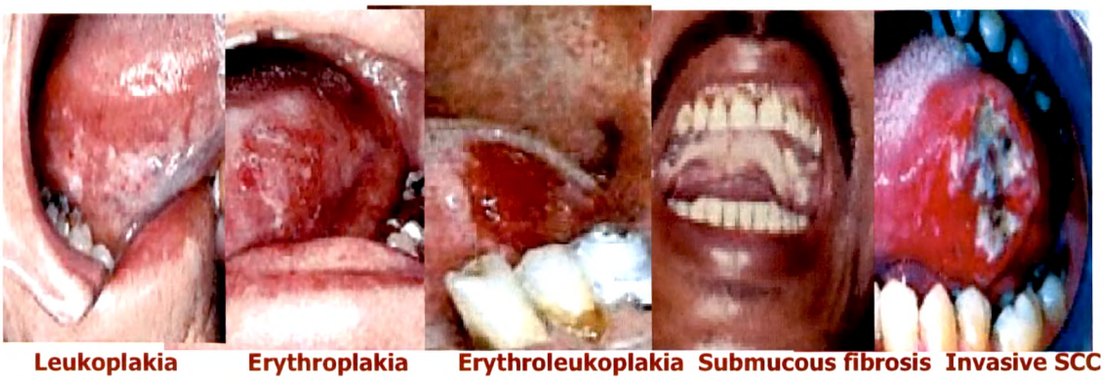
Oral cancer progresses to its malignant phenotype through initial stages, which are termed as pre-malignant (precancerous) lesions causing noticeable clinical and histological changes in the oral mucosa [Silverman, 1968; 1984]. Pre-malignant (precancerous) lesions are also termed as intra epithelial neoplasia (IEN), which is a pathologically discernable intermediate state between normal epithelium and invasive cancer. IEN (dysplasia or precancer)

is characterized by changes in the size and shape of cell nuclei, and increases in the number of cell and abnormalities in cell mitosis. This abnormalities confined by the basement membrane distinguishes this neoplastic state (precancer) from invasive neoplasia (cancer). Most premalignant lesions clinically present as erythroplakia, leukoplakia, erythroleukoplakia or sub-mucous fibrosis (SMF), but histologically, they may have range of phenotypes such as hyperkeratosis, dysplasia or carcinoma. Although common, all these lesions have an unclear etiology, do not have an obvious clinical diagnosis, and cannot be wiped off. Moreover they vary with degree of transformation, making it often difficult to predict which of these shall progress to malignancy [Axell, 1996]. Fifteen percent of premalignant lesions are dysplastic of different grades and 11-36% of dysplastic lesions convert to carcinoma. To clinically manifest the pre-malignant prototypes, the epithelial cells undergo molecular alterations including hyperplasia, dysplasia and finally to a potentially malignant phenotype like carcinoma in-situ to metastasis.

Figure-2.12.1: Pathogenesis of oral premalignant lesions.



Oral premalignant lesions / Intra epithelial neoplasia (IEN) lesions



Leukoplakia **Erythroplakia** **Erythroleukoplakia** **Submucous fibrosis** **Invasive SCC**

The definitive diagnosis of oral cancer can only be determined by histopathological examination of a biopsy specimen of oral tissue. The microscopic diagnosis range from normal, through varying degrees of

dysplasia (mild, moderate, or severe), to carcinoma in-situ to invasive malignancy (**Figure-2.12.1**).

Oral leukoplakia may be defined as a white patch, which cannot be rubbed off and cannot be characterized clinically or histologically as any disease. An estimated 85% of oral premalignant and malignant lesions present clinically as leukoplakias. They are typically found in approximately 1-5% [Bouquot JE, 1991; Bouquot 1988] of adults and mostly in middle-aged or older men and prevalence increases with age [Bouquot, 1994]. The rate of malignant transformation of leukoplakia is estimated to be 3.6-17.5%, occurring on average seven years following initial diagnosis [Kademani 2007; Lee, 2000; Schepman, 1998;]. The American Cancer Society estimates that up to 25% of leukoplakias are premalignant or malignant [American Cancer Society, Available at: http://documents.cancer.org, 2007]. Up to 42% of leukoplakias that contain dysplasia will eventually undergo malignant degeneration [Rhodus, 2005]. Verrucous leukoplakia is an aggressive form found in 80% of women population. It has a high recurrence rate and may spread to multiple oral sites. It has a high rate of malignant transformation (70.3%) to verrucous carcinoma or squamous cell carcinoma [Bagan, 2003; Vigliante, 2003; Saito, 2001; Silverman, 1997].

Erythroplakia is a well-defined red, velvety or granular patch of the mucosa, which is usually irregular in outline. This lesion is easily missed out but is considered to have great malignancy potential. They are relatively rare with reported incidence of 0.2-0.8% occurring most commonly in middle-aged persons [Lumerman H, 1995]. 70% to 90% have been found to be severely dysplastic or frankly malignant at the time of initial biopsy [American Cancer Society. Available at: http://documents.cancer.org/; Neville et al., 2002]. Up to 50% of these lesions are invasive OSCC, 40% are carcinoma in situ, and 9% have mild-to moderate dysplasia. [Mashberg, 1995; Silverman, 1984; Shafer, 1975].

Oral sub mucous fibrosis presents as a loss of elasticity of the oral mucosa with fibrous bands causing limitation of opening of mouth. This condition has

high rate of malignant transformation and is most commonly seen in Asian patients who chew arecanuts along with tobacco.

The next stage after the precancerous lesion is the cancerous lesions. **Squamous cell carcinoma** is an invasive malignancy of oral epithelium. It is the most common type of oral cancer, accounting for more than 90% of all malignant neoplasms of the oral cavity, where 50% patients present in their advanced stage of the disease [Neville et.al., 2002]. It normally starts from any of the precancerous lesion in the mouth. The appearance of squamous cell carcinoma is variable; more than 90% of cases are erythroplakic, and about 60% have a leukoplakic component. A combination of colors and surface patterns, such as a red and white lesion that is exophytic, infiltrative, or ulcerated, indicates instability of the oral epithelium and is highly suggestive of carcinoma. Early lesions are often asymptomatic and slow-growing. As the lesion develops, the borders become diffuse and ragged, and indurations and fixation ensue, in some cases surrounded by leukoplakic or erythromatous changes. If the mucosal surface becomes ulcerated, the most frequent oral symptom is that of a persistent sore or irritation. It may also manifest itself as an exophytic, pappilary or verrucous growth. Verrucous carcinomas are low grade lesions that invade by a pushing front with a better prognosis and low rates of cervical metastasis than invasive squamous cell carcinomas, which have propensity for locoregional metastasis. In terms of tumor differentiation they may range from well, moderate or poor. The oral mucosa might be keratinized or nonkeratinised. With advancing disease, patients may report numbness or a burning sensation, swelling, or difficulty in speaking or swallowing. This cancer is extremely malignant and even if there is slight delay it spreads by local extension or by way of lymphatic vessels of neck. Affected patients may have palpable regional (submandibular or anterior cervical) lymph nodes. These nodes can be large, firm, rubbery, and possibly fixed to underlying tissue. Lesions can extend to several centimeters in diameter if treatment is delayed; this delay permits lesions to invade and destroy vital osseous structures. Once it spreads the prognosis becomes poor and death is inevitable and is because of erosion of major blood vessels and

erosion of the base of the skull, cachexia and secondary infection of the respiratory tract.

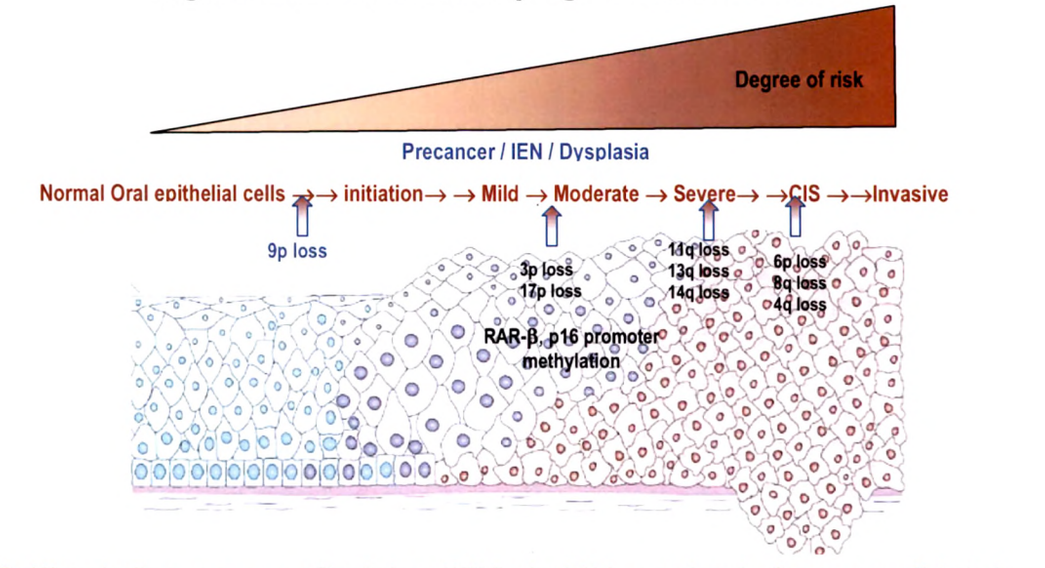
2.13 Biology of oral cancer

The biology of cancer is complex but remarkable advances in multiple fields of cancer research have aided the biologists in better understanding its molecular and cellular pathogenesis as well as its progression. Oral squamous cell carcinomas constitute 90% of all oral cancers that involves abnormal proliferation and progression of a cancerous cell from normal cells of epithelial origin. Epithelial carcinogenesis has been divided into three phases: initiation, promotion, and progression. Initiation may involve transformation of abnormal cells going through phase of hyperplasia, mild to severe dysplasia manifested in the form of precancerous lesion (IEN). In most epithelial cells and tissues, accumulating mutations (that is, genetic progression) and loss of cellular control functions are observed as the phenotype changes from normal histology to early IEN (dysplasia or precancer) then to increasingly severe IEN, superficial cancers and, finally, invasive disease [Renan, 1993]. This condition might develop over approximately 4-10 years, with progression to carcinoma in situ (CIS) clinically significant carcinoma occurring up to 6-8 years later as pictorized in **Figure-2.13.1**. There are many theories that aid in understanding the basic concept of tumour biology.

Oral carcinogenesis proceeds through not specific order but rather accumulation of distinct phenotypic and genotypic steps involving alterations in normal mucosa to dysplastic mucosa to carcinoma in situ to invasive carcinoma [Batsakis,1979]. These molecular alterations are induced via constant exposure of carcinogens in tobacco throughout the entire mucosa of upper aerodigestive tract originating the concept of field cancerization or condemned mucosa in 1953 [Slaughter, 1953]. In oral cancer, tumor is formed by a heterogeneous collection of cancerous cells that acquire characteristics conferring them a selective growth advantage over other neighboring cells as proposed by Nowell's theory of clonal evolution [Nowell,1976]. The degree of genetic damage reflects a composite of

carcinogen exposure and inherent tissue sensitivity. Use of tobacco imposes carcinogenic insults or events occurring at the genetic or epigenetic level progressing and accumulating in mutant progeny that arise as a result of further insults and genomic instability to give rise to cancer. This may confer instability (e.g. aneuploidy) via alterations of the genomic guardian "p53" [Brennan et al, 1995] and the chromosomal region 3p [Wu et al, 2002] acceleration the rate of further genetic alterations during oral tumorigenesis [Shin et al, 2001]. These genetic events may result from numerous cytogenetic alterations, interactions with carcinogens that are implicated in phenotypic progression of cancer like loss of heterozygocity (LOH) of 3, 4, 6, 8, 11, 13, 14 and 17 chromosomes involving either of arms at distinct stages of oral cancer [Califano et al, 1996].

Figure-2.13.1: Molecular progression of oral cancer



Adapted from Nature Reviews. Cancer, 2007

While Fearon and Volgelstein proposed an accumulation of multiple discrete genetic events occurring in the progression of cancer including oncogenic activation, inactivation of tumor suppressor genes leading to development and progression of normal cell growth to neoplasia and tumor formation

[Fearon 1990; Volgelstein 1993]. Being a genetic disease, it involves mutation or alterations in genes normally implicated in cellular functions causing malignant phenotypic changes in cancer. These genes are traditionally classified as either "oncogenes" when activated, get turned on and conversely as "tumor suppressor genes" when deactivated, gets turned off leading to unchecked proliferation and malignant phenotype by modulating a set of thousands of other genes participating in the process of tumor progression, including those involved in angiogenesis, apoptosis, invasion and metastasis. Oncogenes prior to activation are termed as proto-oncogenes and the ones implicated in oral cancer include specific families of cellular oncogenes like int-2, bcl-1, cyclin D1, and C-erb/neu c-myc, ras genes, bcl-2 etc due to aberrant expressions (amplifications or mutations). These families of oncogenes are amplified and activated in the more advanced stages of OSCC, altering and promoting uncontrolled cellular growth, aneuploidy, and tumorigenesis. Tumor suppressor genes are guardians of genome preventing cells from undergoing uncontrolled growth and division. In normal state, these genes act on cell cycle control, transcriptional regulation, or apoptosis, acting as checkpoints to control entry into active proliferative states or causing apoptosis. When they are deactivated, through mutation or other events, the cell loses this negative control and is free to undergo uncontrolled growth and division. A large number of tumor suppressor genes have been identified that are implicated in squamous cell carcinoma of oral cancers including retinoblastoma (RB), p53, p16, PTEN, TGF-β type IIR that are based on paradigm of Knudson's dominant negative model [Knudson, 1971] giving rise to inherited cancer susceptibility. In brief, accumulating molecular, or genetic and epigenetic, alterations within oral carcinogenesis include alterations of tumor suppressor genes such as FHIT (loss of heterozygosity [LOH] at chromosomal region 3p14), p16 (promoter hypermethylation or LOH at 9p21), and p53 (inactivation/loss or mutation at 17p), cyclin D1 overexpression (and gene amplification at 11q13), and telomerase activation [Lippman and Hong 2001; Mao et al, 2004]. All these molecular alterations induced by tobacco carcinogenesis leads to dysregulated proliferation, increasing frequency of

mutations causing genomic instability, invasiveness and metastatic potential as crucial events in oral carcinogenesis.

2.14 Focus on different markers

Bio-molecular alterations are associated within cancerous cells undergoing malignant transformation that are implicated varyingly at different stages of cancer. Cancer is affected by key processes involving either enhancing tumor growth or arrest. Enhancement of tumor is attributed to mechanisms like cell cycle acceleration and proliferation, inflammation, hypoxia, angiogenesis, invasion and metastasis. While, on the other hand tumor suppression is mediated by processes like apoptosis, cell cycle regulatory mechanisms, inhibitory pathways. These mechanistic processes involve biomolecules with varied functions attributed to transcription factors, oncogenes, angiogenesis, inflammation, stress, adhesion and matrix degrading proteases. Besides cells imbibing tumor properties, microenvironment in tumor milieu involving tumor-host interactions also plays critical role in biological activation of key molecules implicated in pathogenecity of oral cancer. Advancement in the analysis of these molecular alterations associated with cancer has laid insight to mechanisms that led to occurrence and progression of malignancies. There has been pile of data emphasizing the aberrant expression of these proteins in cancerous cells. Animal studies have promoted the role as well helped in elucidating the pathways underlying in it. But contrast to animal studies, there are contrasting reports with clinical findings in oral cancer. The clinical behaviour of oral SCC is difficult to predict based on classical histopathological parameters alone. Consequently, the identification of molecular markers that can accurately define those lesions that will manifest an aggressive behaviour and worse prognosis is of pivotal importance. Moreover discovery of novel biomarkers designed to predict the risk of relapse or that could be used as targets of new targeted anticancer agents are clearly needed [Rosset al, 2003]. Characterization of this malignant disease by molecular markers improves our understanding of variations in the clinical course of individual patients and help to estimate their prognosis. In consideration of their

relevant role in human carcinogenesis and of their still unsettled clinico-pathological implications in OSCC, the current study aimed to focus on expression of NFκB, iNOS, Hsp-70, Bcl-2, Bax, Bcl-2, gelatinases (MMP-2, MMP-9) and circulating levels of oxidative stress and antioxidant enzymes, p53 autoantibodies, IL-8, glycoprotein conjugates in serum, gelatinases (MMP-2, MMP-9) and their inhibitors (TIMP-1, TIMP-2) in plasma as key mediators in oral carcinogenesis and their evaluation in oral cancer. These biomarkers are described in brief below. But the results were not uniform and the prognostic value of their expressions remains unclear.

NFκB (Nuclear factor kappa-light-chain-enhancer of activated B cells)

NFκB is a ubiquitous nuclear transcription factor mapped on chromosome 11 q13 that was first identified in 1986 by Sen and Baltimore. It was so named because it was found in the nucleus bound to an 11-base pair sequence in the enhancer element of the immunoglobulin kappa light chain gene in B cells. It represents a group of structurally related proteins, with five members in mammals forming dimers by combination of several proteins including NFkB p65 (REL A) [Baldwin, 1996; Karin et al, 2004]. Consistent with its role in inflammatory and immune responses [Shishodia and Aggarwal, 2004; Nakanishi and Toi, 2005], incorrect regulation of NFκB has been linked to cancer, inflammatory and autoimmune diseases, septic shock, viral infection, and improper immune development. Under normal conditions, NFκB dimers are retained in the cytoplasm of cells remaining bound to inhibitory proteins known as I·Bs which masks the NLS nuclear localization signals within NFκB by aid of unique ankyrin repeat domains [Jacob 1998]. But when stimulated, NFκB is released from I·B inhibition by series of degradative events (phosphorylation, polyubiquitinination and proteosomal degradation) allowing its translocation inside nucleus where it binds with the consensus sequence of target DNA. If NFkB is found to persist in the nucleus, it is referred to as constitutive activation. One of the first genes that NFκB activates is IκBα itself, which transports activated NFκB from the nucleus to the cytoplasm. NFκB activation is therefore an inducible but transient event in normal cells.

While, in tumor cells, different types of molecular alterations may result in impaired regulation of NFκB activation. In such cases, NFκB loses its transient nature and becomes constitutively activated. This leads to deregulated expression of NFκB controlled genes. It modulates transcription of target genes including those implicated in cellular response to various stresses, and those that code for angiogenic factors, cell adhesion molecules, antiapoptotic factors and chemokines, proteases, and also those involved positively in cell proliferation, cell survival, tumour invasion and metastasis. Extensive studies performed on cancer cell lines and preclinical models support a role of NFκB in cancer development and progression [Karin et al, 2002]. Number of studies in human solid tumours and haematological malignancies have also provided clinical data to support the concept that NF-kB may have an important role in vivo in human cancer [Rayet and Gelinas, 1999; Karin et al, 2002]. Its activation seems to be essential for growth and survival of many cancer cells suggested by inhibition studies [Furman et al., 2000; Cahir-McFarland et al, 2002]. The importance of NFκB expression in clinical cancer specimens is highlighted by the current efforts to develop novel drugs that inhibit NFκB activation with the ultimate goal to use them for cancer treatment [Orlowski and Baldwin, 2002; Karin et al, 2004]. In particular, a recent study showed that overexpression of p65, the best characterized NFκB protein, was an independent predictor of poor prognosis in prostate cancer patients [Ross et al, 2004] and its nuclear localization was associated to a high risk of biochemical relapse [Fradet et al, 2004]. Previous studies also demonstrated that suppression of NFκB in cancer inhibits proliferation, causes cell-cycle arrest, and leads to apoptosis, suggesting the important role of NFκB in cell proliferation and survival (Bharti and Aggarwal, 2002). Furthermore, NFκB is known to inhibit apoptosis through the induction of anti-apoptotic proteins, and to suppress the apoptotic potential of chemotherapeutic agents, leading to chemoresistance (Nakanishi and Toi, 2005). Aberrant expression of NFκB proteins has been well-documented in other cancers (Dolcet et al., 2005). Expression of NFκB has been found to be upregulated in OSCC, the level increasing gradually from premalignant lesions to invasive cancer (Mishra et

al., 2006). NFκB is the subject of much active research among pharmaceutical companies as a target for anti-cancer therapy [Escárcega et al., 2007]. Analysis of these data suggests that NFκB activation plays an important role in oral carcinogenesis.

Inducible Nitric oxide synthase (iNOS)

Chronic inflammation is a known risk for oral cancer and it is modulated extensively by a highly reactive free radical named nitric oxide (NO). It is a multifunctional gaseous molecule synthesized from L-arginine, NADPH and oxygen by NO synthase (NOS). As a signalling molecule, NO regulates various physiological and pathophysiological processes, such as vascular functions including angiogenesis, neurological functions and, at relatively high concentration, cytotoxic functions. Interestingly, various studies have also shown that NO can both promote and inhibit tumour progression and metastasis. NO interacts with numerous biological targets and modulates gene expression via effects on transcription factors (NFkB), elements of signal transduction pathways (G proteins etc.), mRNA stability and translation, and DNA methyltransferases. Effects on gene expression can be either activating or inhibitory, even on the same pathway. NO is implicated in modulating several events involved in malignant transformation and cancer promotion/progression. It can directly damage DNA, inhibit DNA repair, block apoptosis, facilitate angiogenesis and enhance oncogenes expression. The effects of NO in tumours seem to depend on the activity and localization of NOS isoforms, concentration and duration of NO exposure, and cellular sensitivity to NO. Three isoforms of NOS are known namely neuronal NOS (nNOS, also known as NOS1), inducible NOS (iNOS or NOS2) and endothelial NOS (eNOS or NOS3). nNOS and eNOS are constitutively expressed (predominantly in neuronal cells and vascular endothelial cells, respectively) and are therefore also referred to as constitutive NOS (cNOS). cNOS activity is dependent on the concentration of cytosolic calcium (Ca2+), which increases upon various physiological stimuli and facilitates the binding of calmodulin to cNOS1. Conversely, iNOS is transcriptionally regulated and induced by

inflammatory cytokines, endotoxin, hypoxia and oxidative stress. iNOS is not dependent on intracellular calcium levels and produces more NO than cNOS [Nathan and Xie, 1993] and largely regulated at the level o synthesis and stability of mRNA and protein. The expression of iNOS is regulated by transcription factors including NFkB. Various upstream signaling pathways are used to enhance or inhibit expression, depending on stimulus and cell type. NO itself affects iNOS transcription; low levels activate NFκB and up-regulate iNOS expression, whereas high levels decrease transcription. iNOS has been associated with the development of human and animal cancers in vivo. It is expressed in various types of cancers [Kro¨ncke et al., 2000]. Once expressed, iNOS produces high amounts of NO for prolonged periods of time which causes nitrosative stress. Induction of the high-output iNOS usually occurs in an oxidative environment and is also known to mediate neoplastic transformation in oncogene- and chemical-induced tumorigenesis models, although conflicting results are reported in the literature. Conversely, the transfection of iNOS-expressing constructs into NO-sensitive tumour cells inhibits tumour growth and metastasis. Many aspects of carcinogenesis like inflammation, angiogenesis are modulated by iNOS expression in tumor as well as stromal cells which is essential for tumour growth and metastasis. Studies have also documented iNOS as tumor suppressor and also as tumor promoter [Xie et al, 2003; Ambs et al, 1998]. Constitutive expression of iNOS is demonstrated not only by cancer cell lines, but equally also by cells of normal mucosa around the tumors [Brennan et al, 2002; Aaltoma et al., 2001]. An increased level of iNOS expression and/or activity has been found in the tumor cells of gynecological malignancies in the stroma of breast cancer and in the tumor cells of head and neck cancer. iNOS is not expressed in normal human oral mucosa but is up-regulated in epithelial dysplasia; expression correlates with severity of dysplasia and is not related to tumor grade in squamous cell carcinomas [Brennan et al., 2000; 2001]. Increased activity correlates with tumor vascularization and is higher at the invasive tumor edge than in the tumor core [Gallo et al., 1998]. Moreover, increased activity of iNOS was found to be correlated with severity of dysplasia in

tumors of oral cavity [Brennan et al., 2000; 2001] while its increased activity also correlates with angiogenesis and metatasis [Gallo et al., 1998] with a promoting effect on neoplasia. Although strong iNOS expression has been correlated to a rapid cancer cell proliferation rate, dedifferentiation and prediction of poor survival [Aaltoma et al., 2001]), the role of iNOS expression with clinical behaviour of oral cancer remains unclear.

Heat Shock protein -70 (Hsp-70)

Cells respond to a variety of stressful stimuli by accumulating and/or activating a set of highly conserved proteins known as heat-shock proteins (HSPs). They have been subsequently characterized as molecular chaperones, which have in common the property of modifying the structures and interactions of other proteins often interact in a stoichiometric manner with their substrates [Beckmann et al 1990; Freeman and Yamamoto 2002]. These molecular chaperones also play a pivotal role in maintaining a delicate balance of cellular homeostasis between survival and death. Among these Hsps, Hsp-70 is one of the most abundant chaperons expressed in mammalian tissues and after stress [Hickey and Weber, 1982; Zhao et al, 2002]. Its expression is regulated by transcription factors belonging to the heat shock factor (HSF) family [Sorger and Pelham, 1988]. The physiopathological features of the tumor microenvironment (low glucose, pH, and oxygen) also tend to induce these proteins and over express them in cancer. Because the elevated Hsp levels associated with malignancy tend to persist when cells are grown in tissue culture, they may well be related to the genetic changes associated with tumor progression (Tang et al 2005). It not only modulates by interacting with house keeping genes like p53 but also affects key apoptotic proteins like Bcl-2 and Bax in cancerous cells, thereby might affect treatment response by interfering with the apoptotic functions in tumor cells. Hsp-70 exerts its inhibitory effect downstream of the release of cytochrome c and upstream of activation of caspase-3 [Li et al, 2000]. It inhibits apoptosis by binding directly to Apaf-1, the eventual recruitment of procaspase-9 to the apoptosome [Beere et al, 2000; Saleh et al, 2000]. Overall human tumor

biopsies as well as cell culture and animal experiments have indicated dual role of Hsp-70 in cancer. One is promoting cancer development by suppression of various anti-cancer mechanisms like apoptosis and senescence as well as by facilitating expression of metastatic genes and the other is by facilitating tumor rejection by immune system. Over expression of Hsp70 can be used as diagnostic or prognostic marker [Wong et al, 2005] or used for assessing progression, lymph-node metastasis in cancer patients [Noguchi et al, 2002]. Chuma et al (2003) propagated Hsp70 as the most abundantly upregulated gene in early hepatocellular carcinoma and suggesting it as sensitive marker for the differential diagnosis of early hepatocellular carcinoma. Studies have indicated significant positive correlation of Hsp70 with axillary lymph-node metastasis [Lazaris et al, 1997]. Elevated levels are reported in various tumors especially of epithelial origin [Nanbu et al, 1998 ; Jäättela et al, 1999]. Moreover their expression is also found to be correlated to increased cell proliferation, lymph-node metastases, poor response to chemotherapy and poor survival [Jäättela et al, 1999; Ciocca et al, 2005; Calderwood et al, 2006]. Hsp70 expression is correlated with poor prognosis in breast cancer, endometrial cancer, uterine cervical cancer, and transitional cell carcinoma of the bladder. This is consistent with the Hsp70 associations with poor differentiation, lymph node metastasis, increased cell proliferation, block of apoptosis, and higher clinical stage, which are markers of poor clinical outcome. In contrast, high Hsp70 expression was correlated with good prognosis in oesophageal cancer, pancreatic cancer, renal cancer, and melanoma. Hsp70 expression showed no correlation with prognosis in ovarian cancer, oral cancer, head and neck squamous cancer, gastric and prostate cancer, and leukemia. However, one recent study indicates that when cells are transferred from tissue culture to growth as xenografts in vivo, Hsp expression declines markedly (Tang et al 2005). Increasing levels of Hsp-70 correlated with carcinogenesis, clinical stage and poorly differentiated SCC [Kaur et al., 1998]. Hsp70 has been involved not only with poor tumor differentiation (breast, ovary, and oral epithelium) but also with increased cell proliferation (breast, uterine cervix, lung), lymph node metastasis (breast,

colon), increased tumor size (uterine cervix), presence of mutated p53 (breast, endometrium), and higher clinical stage (oral, colon, melanoma). In addition, Hsp70 has been associated and complexed with mutant p53 in cancer cell lines (Lehmann et al, 1991). As Hsp-70 expression is known not only to be correlated with the carcinogenic process as well as with the degree of differentiation and cell proliferation, but also implicated in the regulation of apoptosis. Therefore, it was reasonable to study the Hsp-70 in oral cancer patients.

Apoptotic related proteins (Bcl-2 and Bax)

Programmed cell death also termed, as apoptosis is the necessary mechanism complementary to proliferation that ensures homeostasis of all tissues. This process needs to be highly regulated since defects in the apoptotic machinery will lead to extended cell survival and may contribute to neoplastic cell expansion, extended cell survival also creates a permissive environment for genetic instability and accumulation of mutations. Furthermore, defects in apoptotic pathways confer resistance to chemotherapy, radiation and immune mediated cell destruction. In order to prosper, it is requisite for the cancerous cells to evade or develop resistance to apoptosis as well shift the balance between cell division and cell death. It is a gene-directed program regulated by many genes in which some act to induce and others act to suppress cell death. It is triggered or induced by stimulus like ROS, stress, including cytotoxic insults like cytokine deprivation, UV- and -irradiation, heat, deregulated oncogenes and chemotherapeutic drugs. In cancerous cells, this program is not absent but is either malfunctioning or insufficient to remove the genetically damaged transformed malignant potential cells. One of the best studied and characterized regulatory molecules in the apoptotic pathway include BCL-2 family of proteins with 17 or more members in mammalian cells [Cory and Adams, 2007, 2002]. Proteins of bcl-2 family form heterodimers and inhibit each other's activity. The dimerization is influenced by phosphorylation of the amino acid residues of the proapoptotic members, Bax, Bak, and Bik. The expression level of each Bcl-2 family protein is

controlled by transcription, heterodimerization, and ubiquitination. Phosphorylation of antiapoptotic Bcl-2 family proteins inhibits the binding of these proteins and polyubiquitin. They serve as "life/death switch" determining whether or not the apoptotic pathway should be activated to decide the fate of a cell. They are situated upstream of irreversible cell damage in the apoptotic pathway providing a pivotal decisional checkpoint in the fate of the cell after a death stimulus serving as sensors and checkpoints for life-or-death decisions.

B-cell CLL/lymphoma 2(Bcl-2)

Bcl-2 is the first oncoprotein or oncogene acting as antiapoptotic protein i.e., enhancing cell survival by inhibiting apoptosis even in adverse conditions [Gross et al, 1999; Rudin et al, 1997]. It was originally discovered in 1988 in human follicular lymphomas because of its involvement in the t(14;18) chromosomal translocation in which bcl-2 gene is translocated into the immunoglobulin (Ig) heavy chain locus resulting in its massive overexpression and activation in B cells and hence was named as bcl [Tsujimoto et al , 1985; Cory and Adams, 2007, 2002]. Mutations in the Bcl-2 gene can contribute to cancers. Apoptotic mechanisms are compromised by deregulation of the anti-apoptotic influence of Bcl-2. The gene for bcl-2 is encoded on chromosome 18q21 at locus 33 and at 3 and frequently involved in t(14;18) (q32:q21) in follicular lymphomas. Its product is an integral inner membrane protein of 25 kDa with either 205 amino acids (BCL2b) or 239 amino acids (BCL2a, which has, in addition, a hydrophobic tail for membrane anchorage; this tail seems necessary for anti apoptotic ability). It belongs to Group-I of Bcl-2 family including other anti-apoptotic proteins like Bcl-xL protecting cells from death. Though mainly localized in the mitochondrial membrane; it is also a component of the nuclear envelope, endoplasmic reticulum and outer mitochondrial membrane [Kannan et al, 1998]. It has a wide tissue distribution and found in B and T cells in particular. The anti-apoptotic function of Bcl-2 is regulated through proteolytic processing and phosphorylation. Overexpression of bcl-2 in genetically modified cells, such

as tumour cells, contributes to the expansion of the damaged cells clone by promoting cell survival and by preventing cell turnover due to apoptosis, leading to cellular immortalization [Johnstone et al, 2002] and its increased expression of the bcl-2 protein can be detected in about 50% of human cancers, further emphasizing the importance of deregulated apoptosis as a fundamental step in human carcinogenesis. In fact, by promoting cell survival, bcl-2 facilitates the permanent acquisition of mutations and malignant transformation [Vaux DL et al, 1988]. Moreover, increased bcl-2 expression in cancer cells possibly reflects tumour cell resistance to apoptosis and may have implications for their responsiveness to treatments [La Casse et al , 1998]. Along with other Bcl-2 family members it forms complexes with caspase-9 and APAF1 which prevent them to initiate the protease cascade (through caspase-3 cytochrome C dependent activation) leading to apoptosis. Bcl-2 is a prosurvival multidomain protein that regulates apoptosis by preventing the release of proapoptogenic factors from the mitochondria (e.g., cytochrome c) and subsequent caspase activation [Cory S et al, 2003; Gross A et al, 1999]. In addition to promoting cell survival, Bcl-2 has been implicated in the differentiation of several cell types, including neuronal, epithelial, and hematopoietic cells [Haughn et al, 2003]. Consequently, Bcl-2 has become a very attractive target for the design of new anticancer drugs. Some studies have also propagated Bcl-2 as a proangiogenic signaling molecule through its ability to activate the NFκB signaling pathway and to induce expression of the proangiogenic CXCL8 and CXCL1 chemokines in endothelial cells that is independent on its ability to enhance endothelial cell survival [Elisabeta etal, 2005]. It is also known that Bcl-2 induces matrix metalloproteinase-9 (MMP-9) through a NFκB –dependent pathway in breast cancer cells [Ricca et al, 2000]. Overexpression of Bcl-2 is not limited to B cell lymphomas, but is a common feature in several human cancers including other hematopoietic malignancies as well as several other tumors including carcinomas of breast, prostate, ovary, lung, neuroblastomas, colorectal, thyroid, kidney, nasopharynx without any apparent gross chromosomal rearrangement [Kannan et al, 1998; Reed, 1997, 1994]. Bcl-2 expression in human cancers

has been expected to correlate with poor prognosis. Surprisingly, this is the case only in a subset of cancers including certain types of leukemia, lymphoma, and prostate cancer, whereas Bcl-2 expression in adenocarcinoma is most often a marker of increased survival and in some cases even of better response to therapy. Over production of bcl-2 protein has been shown to be associated with resistance to some chemotherapeutic agents and this may be a reason for poor prognosis of bcl-2 expressing patients [Miyashita et al, 1992]. The bcl-2, through extended cell survival, can facilitate the acquisition of additional mutations in other tumor suppressor genes and oncogenes, which cumulating results in clonal progression. Hence, Bcl-2 is a critical cellular factor contributing to the pathogenesis and progression of cancer.

BCL2-associated X protein (Bax)

One of the most potent downstream effector molecule associated with the induction of apoptosis is BCL2-associated X protein. The gene is mapped onto chromosome 19 at locus q13.3-q13.4 and also known as Bax zeta. It is transcribed alternatively and spliced to give multiple transcripts variants which encode 5 different isoforms namely BCL2-associated X protein isoform alpha, beta, delta, epsilon and sigma. The protein encoded by this gene belongs to the BCL2 protein family with a M.Wt of 21 kDa. It is a soluble monomeric protein diffusely distributed in cytoplasm. This protein forms a heterodimer with BCL2, and functions as an apoptotic activator. Bax belongs to group II of Bcl-2 family members with pro-apoptotic activity. Members of this group, which includes Bax and Bak, have a similar overall structure to group I proteins, containing the hydrophobic tail and all but the most N-terminal, BH4 domain [Adams and Cory, 1998]. Diverse apoptotic stimuli induces a conformational change in Bax unmasking specific epitopes needed for membrane insertion, thereby facilitating its translocation to the outer mitochondrial membrane and oligomerization [Rashmi 2005; Suzuku,2000; Mkin et al., 2001], eventually it forms death pores that faciliate the release of cytochrome C and other proapoptotic activators from the mitochondrion. Two amino acids, leucine-70 and aspartate-71, within the BH3 homology domain

of Bax play a critical role in regulating its multimeric state i.e dimerization, localization, mitochondrial dysfunction, caspase activation, and apoptosis [Wang K, 1998] which are not associated with direct effect on cytochrome c release [Jurgensmeier, 1998]. Bax was isolated as a protein that formed a complex with Bcl-2 protein and its proapoptotic function was evident by the vector-transfection studies on interleukin-3-dependent cell line transfected with Bax expression vector sensitivity to apoptosis [Oltvai, 1993]. This protein interacts and increase the opening of, the mitochondrial voltage-dependent anion channel (VDAC), which leads to the loss in membrane potential and the release of cytochrome c. Bax along with other constitutive mitochondrial protein, adenine nucleotide translocator, cooperates within the permeability transition pore (PTP) complex to increase mitochondrial membrane permeability, activate caspases and trigger cell death [Marzo, 1998]. It is presumed that the apoptotic signal induced by Bax overexpression is mediated by mitochondrial cytochrome c. The expression of this gene is regulated by the tumor suppressor P53 and has been shown to be involved in P53-mediated apoptosis. Data suggest that Bcl-2 functions as an inhibitor of mitochondrial permeabilization by changing conformation in the mitochondrial membrane to bind membrane-inserted Bax monomers and prevent productive oligomerization of Bax. Bcl-2 interacts with activated Bax during apoptosis in an effective manner to neutralize the proapoptotic activity of Bax. p53 accumulation and loss of bax expression influence the acquisition of a malignant phenotype but seem to have no further impact on tumor progression. p53 directly activated the proapoptotic Bcl-2 protein Bax in the absence of other proteins to permeabilize mitochondria and engage the apoptotic program. Hsp70 blocks heat-induced apoptosis primarily by inhibiting Bax activation and thereby preventing the release of proapoptotic factors from mitochondria. Overexpression of Bax has been shown to induce apoptosis in wide variety of cell lines including prostrate, colon, cervical and ovarian cancers [Pirocanac, 2002]. Homodimers and heterodimers formed by proteins of this family either promote or inhibit the process of apoptosis. Conversely bcl-2 binds to bax and can form heterodimers, thereby inhibiting

the bax activity. Thus the ratio of bcl-2 to bax protein has been reported to be correlated with apoptosis in tumour cells [Masahide Ikeguchi et al, 2002; Oltvai Z, 1993].

Gelatinases and their tissue inhibitors

One family that has been shown over years to play pivotal role in tumor cell invasion and establishment of metastatic deposits by proteolysis of ECM is matrix metalloproteinases (MMPs) family [Stetler - Stevenson, 1993, Matrisian,1992]. At least 20 different MMP family members (zinc-containing endoproteinases) have been identified, including collagenases (MMP-1, -8, and -13), gelatinases (MMP-2 and -9), stromelysins (MMP-3 and -10), and MT-MMPs (i.e., MMP-14, -15, -16, and -17). These MMPs are also associated with degradation, remodeling, and release of growth factors from the ECM, stromal invasion and subsequent metastasis as key steps in the progression of malignant tumors. Activation of these proteases is the key step in metastasis. This is the result of complex, multifactorial processes that include the transcriptional control and activation of proenzymes, as well as production of their natural inhibitors. Under physiological conditions, their local activities are tightly controlled in large by endogenous tissue inhibitors of metaloproteinases (TIMPs) that play significant role in regulation and suppresssion of malignant progression [Denhardt et.al., 1993]. Disruption of this balance between MMP's and TIMPs leads to an accelerated breakdown of the ECM and consequently loss of tissue integrity. Overall, it is the net balance between these proteases and their inhibitors determines the net MMP activity that is critical to their effect on the environment and is also a pivotal determinant of ECM turnover.

Gelatinase –A (MMP-2)

Matrix metalloproteinase-2 (MMP-2), also known as the 72 kDa gelatinase/type IV collagenase and gelatinase A (E.C. 3.4.24.24), cleaves a number of substrates including gelatins, collagen types I, IV, V, VII, X, XI and XIV, fibronectin, elastin, and proteoglycan. It is synthesized as a 72 kDa proenzyme that is proteolytically processed to the 66 kDa active form.The

pro-MMP-2 is activated in vitro by certain organomercurials (e.g.,APMA). Pro-MMP-2 and pro-MMP-2/TIMP-2 complexes are specifically activated in vivo by active MT1-MMPs on the surface of tumor cells.A correlation between collagenase expression and metastatic potential has been established and MMP-2 is considered to be a useful marker for the diagnosis or prognosis of cancer.

Gelatinase–B (MMP-9)

Matrix metalloproteinase-9 (MMP-9), also known as gelatinase B and 92 kDa gelatinase/type IV collagenase (E.C. 3.4.24.25), exhibits a broad range of substrate specificity for native collagens including types IV, V, VII, and X as well as gelatin, proteoglycans, and elastin. It is secreted as a 92 kDa proenzyme and can be activated in vitro by certain organomercurials, trypsin, and α-chymotrypsin and in vivo by cathepsin G and MMP-3. MMP-9 can be inhibited by TIMP-1 which binds exclusively to pro-MMP-9 (Kd ~35 nM).

Tissue inhibitors of metalloproteinases (TIMPs) are important regulators of MMP activity. The TIMP family consists of: TIMP-1, TIMP-2, TIMP-3 and TIMP-4, which are differentially expressed in tissues and temporally follow the influx of MMPs. The imbalance between MMPs and TIMPs is considered to be important in the degenerative process. The two gelatinases are unique among MMPs since they are bound with TIMPs in their latent and activated forms; the proMMP-9 with TIMP-1 vs. the proMMP- 2 with TIMP-2. Binding of the TIMPs to their specific MMPs results in the inhibition of MMP activity. TIMPs have been shown to bind to the proenzyme forms of MMP-2 and MMP-9 with high degree of specificity. This interaction provides an extra level of regulation by preventing activation. TIMPs are known to inhibit invasion and metastasis in animal models and, hence, are capable of altering the metastatic potential of cancer cells. TIMPs not only directly control the activity of active MMPs, but also have substantial influence on the activation process of MMP zymogens.

Tissue inhibitor of metalloproteinase-1 (TIMP-1)

TIMP-1 is a 184 amino acid glycoprotein of 28.5 kDa, exhibits a 41% sequence homology with the non-glycosylated 21.5 kDa TIMP-2. TIMP-1 binds to the pro-MMP-9 (Kd ~35 nM). It is more widely distributed than the other TIMPs and inhibits the activity of all the active MMPs. Its binding to MMP- 9 and MMP-1 occurs via a reversible non-covalent binding to the catalytic domain of the MMP protein. (Kd ~10-10 M). TIMP- 1 is not cleaved by this binding and can be recovered with full activity from complexes with MMP-3.it is expressed from the several tissues of organisms.it is is a stromal factor with multiple functions. Overexpression of TIMP-1 correlates with aggressive clinical behavior of a spectrum of tumors. Besides MMP inhibition, it also possess anti-apoptotic, erythroid potentiating and cell growth-promoting activities. It is highly inducible in response to cytokines and hormones. Enhanced TIMP-1 expression is associated with poor clinical outcome in many cancer types [Nakopoulou et al., 2002; Holten-Andersen et al., 1999].

Tissue inhibitor of metalloproteinase-2 (TIMP-2)

TIMP-2, a 194 amino acid unglycosylated protein of 21.5 kDa, has 41% and 44% sequence homology to TIMP-1 and TIMP-3, respectively. It inhibits the activity of all active MMPs and regulates the activation of pro-MMP-2 by binding to its C-terminal region (Kd ~5 nM). TIMP-2 binds to the pro-MMP-2 (Kd ~5 nM). As with TIMP-1, TIMP-2 also exhibits erythroid potentiating activity and cell growth-promoting activities.

Elevated MMP levels in biological fluids, including serum, plasma, and urine from animals bearing experimental tumors or from cancer patients have been reported in several studies [Zucker et al., 1994; Nakajima et al., 1993]. In addition, elevated plasma levels of TIMP-1 and TIMP-2 have been detected in patients of late stage breast, colorectal, lung, and gastric cancer [Holten-Andersen et al., 1999; 2000; Ylisirnio et al., 2000; Yoshikawa et al., 1999; 2000].

Serum p53 autoantibodies

p53 is a tumor suppressor gene, located on chromosome 17p13.1, which plays a role in cell-cycle progression, cellular differentiation, DNA repair, and apoptosis. A major function of p53 is to serve as a guardian of the genome. Endogenous or exogenous stresses, such as DNA damage, hypoxia, and oncogene activation, increase p53 levels, leading to cell-cycle arrest that enables DNA repair to occur (Hartwell and Kastan, 1994). But disruption of the p53 pathway leads to intense genomic instability and trigger carcinogenesis marking an early event in oral cancer. p53 is the most commonly mutated gene and is altered in ~ 50% of all cancers, including 25-69% of oral cancers (Levine et al., 1991; Boyle et al., 1993; Caamano et al., 1993; Baral et al., 1998). Mutation most often occurs at a 'hot spot' region from codon 238 to codon 248 (Somers et al., 1992; Hainaut et al., 1998; Kropveld et al., 1999) and causes defects in the binding of specific DNA sequences and the transactivation of genes whose expression is up-regulated by the wild-type protein (Vogelstein et al., 2001). Such "gain of function" activities include the ability to transform cells, increase tumorigenicity, and modulate the sensitivity of cancer cells to drugs (Sigal and Rotter, 2000; Song and Xu, 2007). p53 mutations commonly arise as a result of alcohol and/or tobacco exposure, and their presence is associated with the early recurrence and development of second primary tumors (Shin et al., 1996). This abnormality almost always leads to synthesis of a structurally and functionally changed protein which is more stable than normal wild-type protein. The consequence of these processes is a stimulation of immunological response expressed by the production of specific antibodies. Its disruption is due to alterations attributed to either mutations, loss of heterozygosity or interaction with viral proteins. Mutations in the p53 tumor-suppressor gene are found at a high frequency in a wide variety of primary human cancers. In cells and tissues with wild-type p53, the protein is difficult to detect. But in cancer the mutant proteins are degraded less rapidly than wild-type p53, which has a half-life of 20mins making them easily detected readily in tumors. p53 alterations have been reported in about 50-90% of head and neck squamous

cell carcinoma [Ahomadegde et al, 1995; Boyle et al, 1993]. In the present study, serological analysis was used to detect antip53 autoantibodies in serum of cancer patients with an immune response against an abnormally high level of p53 protein inside the tumour cells. The serum antip53 antibodies are indirect results of p53 gene missense point mutations. In head and neck squamous cell carcinoma significant association has been observed between anti p53 antibodies and poor clinical outcome, i.e increase risk of relapse and death [Bourhis et al, 1996]. Ralhan et al (1998) have also suggested potential usefulness of p53 antibodies in tobacco and betel quid abused populations for identifying high risk individuals. They reported antip53antibodies as a surrogate marker for early p53 alterations, which can be a potential aid in early detection of oral cancer.

Interleukin-8 (IL-8)

It is also known as a potent pro-angiogenic, neutrophil chemotactic and activating factor. It is a primary inflammatory cytokine produced by many cells (including monocytes/macrophages, T cells, neutrophils, fibroblasts, endothelial cells, keratinocytes, hepatocytes, astrocytes and chondrocytes) in response to pro-inflammatory stimuli such as IL-1, TNF, LPS and viruses. Its function is, in part, to attract neutrophils to the site of inflammation and to activate them. [Oppenheim, 1991; Hack, et al. 1997]. IL-8 has taken a centre stage among the pro-angiogenic chemokines in cancer. It was discovered on basis of its activity serving as leukocyte chemoattractants playing pivotal role only in Immune system but also implicated in several inflammatory associated diseases, including cancer. it is an secreted protein with a molecular weight of ~11kDa. It is produced by many types of cells and possesses a high anti-inflammative property. The basic bioactivity of IL-8 is the induction of chemotaxis of neutrophils, eosinophils, basophils and other cells from immune system. They stimulate growth of cancer cells and contribute to locoregional relapse as well as metastasis. Permanent synthesis and release of these cyto-kines leads to augmentation of their serum concentration that might be utilized as a marker in prognosis and monitoring of the course of cancer.

There has been increasing evidence for role of IL-8 in stimulating tumor angiogenesis and controlling proliferation and metastasis of tumor cells. IL-8 one of the potent pro-inflammatory as well as pro-angiogenic molecule is known to mediate the monocyte adhesion to endotheilal cells via NF•B mediated upregulation. Moreover endotheilal cells are known to release IL-8 in response to VEGF stimulation through BCL2 and or nuclear factor NF•B. The regulatory region of the IL-8 gene contains several potential transcription regulatory elements [Hirani et al,2001]. Three transcription factors in particular, NFκB [Roger et al, 1998], C/EBP and AP-1 [Roebuck et al, 1999] have been implicated in regulating IL-8 gene transcription [Frank Antonicelli et al, 2002]. It plays an important role in tumor angiogenesis and contributes to the aggressive biology of human pancreatic cancer [Le X et al,ref]. It has been shown to be a potent angiogenic factor. Transfection of IL-8 into tumor cells produeces a rapidly growing and highly vascularised neoplasm as compared to control cells [Takamori et al,]. It has recently been shown to contribute to human cancer progression through its potential functions as a mitogenic, angiogenic, and motogenic factor [Xie]. Low tumor pH contributes to the enhanced expression of IL-8 and plays an important role in tumor progression [Shi et al]. It plays an important role in tumor angiogenesis and contributes to the aggressive biology of human cancer. High levels of IL-8 are expressed in tumors of skin, head and neck, breast, ovary, brain and non-small cell lung cancer.

Serum glycoprotein constituents

Malignant transformation of cancerous cells involves molecular changes associated with altered cell surface oligosaccharide component of glycoconjugates including glycoproteins and glycolipids as the hallmark of cancer progression. Being the major component of cell membrane, various glycoprotein constituents are markedly elevated during malignant process. There is evidence that being attached to the surface of tumor cells, these sialoglycoproteins affect various important functions. Further more, expression of proteases and surface proteins enables the tumor cells to mask

immune response enables cancerous cells to invade and metastasize. Altered glycosylation of glycoconjugates is one of the important molecular changes that accompany malignant transformation. Sialic acid, the end moieties of the carbohydrate chain of glycoconjugates are reported to be elevated during malignancy. Increased levels of sialic acid in cancer patients can be explained by spontaneous release (shedding) of aberrant sialic acid rich glycoproteins and glycolipids. Sialic acid moiety of carbohydrate epitope is important for biological interactions including cell adhesion to selectin and lectins. Thus, sialic acid is an important constituent for the characteristic changes of transformed cells. Sialylation is one of the most common and versatile type of terminal glycosylation. The presence of extra sialic acid in glycoproteins of malignant cells has also been demonstrated. Structural analysis of tumour associated carbohydrate antigens has shown that sialylated derivatives together with related structural changes are essential carbohydrate epitopes associated with malignant transformation. Moreover, majority of serum proteins are glycosylated. When disease is present, subtle changes occur in glycosylation of these proteins. Alterations in circulating levels of several glycoprotein constituents e.g. total sialic acid (TSA), lipid bound sialic acid (LSA) and seromucoid fraction [mucoid protein (MP) and hexoses] are implicated at various stages of oral cancer.

Antioxidant enzymes and oxidative stress related biomarkers.

Use of tobacco in any form imposes lethal effects attributed to free radicals (ROS and RNOS) causing oxidative and nitrosative stress that play critical role in initiation and promotion of oral carcinogenesis. It is known to contain various carcinogenic products like polyphenols, alkaloids, nitrosamines etc. with clastogenic and carcinogenic properties [Nair et al., 2004]. Autooxidation of these harmful products in tobacco eventually affects directly onto oral mucosa causing mutagenic and genotoxic damage implicated in oral pathogenesis. Oxidative stress induces a cellular redox imbalance which has been found to be present in various cancer cells compared with normal cells; the redox imbalance thus may be related to oncogenic stimulation. Human

cells generally function in a reduced state but oxidative stress results into imbalance towards more oxidized state resulting into lower levels of oxidized antioxidants [Halliwell, 2000]. As the tumor progresses in these cancer patients, a more oxidized condition prevails within oral tissues with reduced antioxidant capacity [Bounous and Molson, 2003;]. Moreover, a reduced oxidized state is indicated by lower thiol (GSH) levels in cancer patients attributed to oxidative processes elicited by free radicals which in turn reflect the redox state of the cancerous cells. Human body's defense system comprises array of detoxifying enzymes involved in detoxifying the lethal effects of ROS mediated oxidative damage incurred in cancer patients. They are phase-1 and phase-2 enzymes involving antioxidant and detoxifying enzymes. The enzymes involved in the phase-1 as first line of defense against free radical damage in cancer like SOD, Catalase, Gpx, GR. They eliminate and reduce harmful effects of free radicals and other toxic carcinogens by oxidizing them and converting into reactive metabolites that have implications in causing altered membrane permeability, DNA adducts and mutations that may gradually lead to neoplastic transformation. While those involved in phase-2 as second line of defense are GST playing a central role in pathogenesis as well prevention of cancer. GSTs are a superfamily of enzymes that detoxify xenobiotics by conjugating them to glutathione and increasing their cellular excretion. In response to reduced efficiency of first line of defense enzymes and significantly reduced thiol levels or an higher oxidative stress evokes the counter action of enzymes implicated in phase -2 as second line of defense involving GSH depleting and replenishing enzymes GST and GR respectively to fight against free radical scavenging system in cancer. Depletion or variations in these enzymes in various malignancies have been reported [Cook et al., Rawal et al., 1999] which have potential implication to serve as biomarkers useful in diagnosis, prognosis and treatment monitoring of cancer patients.

The current study has therefore focused on studying proteins involved in mechanisms regulating the important cancer phenotypes of altered cell

proliferation, apoptosis and invasiveness. This shall aid not only in-depth assessing these bio-molecules for clinical utility but also in developing mechanism-based strategies for cancer prevention and treatment. Considering the importance in cancer, objectives of the study was to analyze following different bio-molecules for clinical utility in oral cancer.

- To evaluate the status of activation of NF-κB and expression of iNOS, hsp-70, bcl-2 and bax in malignant and adjacent normal tissues of oral cancer patients.
- To evaluate alterations in serum levels of antioxidants and detoxifying enzymes like GST, GR, SOD, Catalase and Thiol in oral cancer patients and controls.
- To evaluate alterations in glycoprotein constituents like TSA, LASA, MP, Hexoses, anti p53 antibodies, IL-8, gelatinases; MMP-2 and MMP-9 as well as their inhibitors; TIMP-1 and TIMP-2 in healthy individuals, oral cancer patients and to assess their clinical usefulness.
- To determine association of above bio-molecules with clinical and pathological variables like age, sex, tobacco habit, tumor size, tumor differentiation, lymph-node metastasis, lymphatic response, tumor infilteration, stage of the disease in oral cancer patients.