CHAPTER 1

INTRODUCTION

About thirty three years ago, US President Richard Nixon declared the "war on cancer" in 1974 with the hope and anticipation that death from cancer might be greatly reduced by the end of last century. In spite of advances in cancer research and treatment, it still remained a major global health issue. In 2000, cancer accounted for over 7 million deaths and there were more than 10 million new cases worldwide by the end of new millennium (Shibuya et al., 2002). According to report of American Cancer Society on cancer statistics, cancers of the lung, prostate, colon and rectum in men and cancers of the lung, breast, colon and rectum in women continue to be the most common causes of cancer deaths (Jemal et al., 2002). These four types of cancer account for more than half of total cancer deaths among men and women.

Carcinogenesis is a complex, multi-step process in which signal transduction pathways involved in various normal cellular physiology are quantitatively or qualitatively altered (Vogelstein and Kinzler, 1993). Under normal conditions, these tightly controlled excitatory or inhibitory pathways regulate cellular functions like cell division, differentiation and senescence.

Although the cellular pathway of different types of cancers may be diverse, they contain the same fundamental elements. An extra cellular ligand, such as a growth factor binds to a cell surface receptor. The receptor-ligand complex generates excitatory or inhibitory signals, sent through intracellular and nuclear messengers that can either directly alter cell function or can stimulate the transcription of genes whose proteins effect change (Bishop, 1991). Cancer is the
result of accumulation of changes in the excitatory or inhibitory cellular pathways that can occur at any level of a given pathway. As the cell collects these alterations or mutations; it becomes functionally independent from the surrounding cells. The normal cellular functions tightly controlled by the stimulatory and inhibitory pathways are subverted in the tumor cell, allowing it to divide more rapidly, sequester blood vessels to feed that growth, delete or amplify signals to produce abnormal structural or functional changes, and invade normal tissue at local or distant sites (Weiner and Cance, 1994).

It has long been evident that cancer has a multi-factorial etiology and is a multi-stepped process involving initiation, promotion and tumor progression. Chemical carcinogens, physical agents, ionizing radiation, viruses and other agents have all been implicated, and clearly host factors are also involved, mainly via an immunological and/or genetic basis. Cancer-predisposing genes may not act only via immune surveillance systems affecting the host's ability to recognize and eliminate incipient tumors, but also may affect the ability to repair damage to DNA or might affect the rate of metabolism of pre-carcinogens or carcinogens.

According to famous British pathologist, R. A. Wills (1960), tumor can be defined as, "An abnormal mass of tissue, the growth of which is in excess and uncoordinated with the normal tissue and persists in the same excessive manner, even after the cessation of the stimuli that evoked the change." The six major alterations in cell physiology that collectively dictate malignant growth (Hanahan and Weinberg, 2000) are:-

Self-Sufficient in growth signals
• Insensitivity to growth inhibitor signals
• Evasion of apoptosis (programmed cell death)
• Limitless replicative potentials
• Sustained angiogenesis and adjoining tissue invasion
• Metastasis

Each of these physiological changes acquired during tumor development represent the successful breaching of an anticancer defense mechanism. These six features are shared in common by most types of human and animal tumors.

Cancer may be caused by a variety of causative factors, usually over a period of many years. Many specific causes of cancer are now known, the most important being difference in lifestyle, occupational exposure and exposure to oncogenic viruses, but a large proportion of global variation for human cancers remains still unexplained. The known risk factors for cancer can be broadly divided into 'Environmental' and 'Host factors'. Environmental factors can be further subdivided into "chemical, physical and biological".

In this era of modernization and technology, large numbers of chemicals enter into our environment through occupational exposure, automobile exhausts, pesticides, industrial wastes and contamination of food and water. These chemicals exist in the environment in a relatively stable condition until taken in by an exposed individual and activated. Thus, these chemicals appear to pose a great concern and threat to man. Polycyclic Aromatic Hydrocarbons (PAH), nitrosamines, mycotoxins, pesticides and metallic contaminants such as arsenic
and cadmium are some of xenobiotics that can enter in our food chain during processing, storage, preservation and cooking. According to International Agency for Research on Cancer (IARC), now there are about 88 chemicals, with sufficient evidences of carcinogenic potential in humans (IARC, 1994). Physical agents such as ultra-violet rays (UV) and the ionizing radiations (X-rays, gamma-rays) are known to be hazardous in nearly all tissues or organs of human or experimental animals depending upon the radiation dose and exposure schedule (IARC, 1992). The scenario is becoming alarming with the application of nuclear technology in science, medicine, and industries and expanding use of these radiations in diagnosis and therapy of the disease itself (Trichopoulos et al., 1996).

Biological agents, such as retroviruses, bacteria and parasitic infections, the oncogenic viruses such as papillomas-viruses, feline-leukemia viruses and bovine –leukosis virus are linked with increased incidence of various types of neoplasia. The Epstein-Barr virus has shown to be implicated with the occurrence of Burkitt's lymphomas, Hodgkin and non-Hodgkin lymphoma and pharyngeal carcinoma (Cordova Perez et al., 2003; Young and Murray, 2003). Certain bacteria like *Helicobacter pylori* are also implicated in the development of cervical, esophageal, head, neck and stomach cancer (Peto, 2001).

Various factors that can play crucial role in establishment of cancer are host (internal) factors comprised of hormonal disturbances, immune functions and inherited predisposition to certain cancers. The genetic manipulations such as activation of cellular proto-oncogenes and alterations in tumor suppressor genes
also result in aberrant proliferation of cells (Ames et al., 1995; Peto et al., 2001). Many cancer chemotherapeutic drugs, particularly vinca alkaloids, alkylating agents, immunosuppressive agents and certain cyclosporins are also reported to increase the cancer risk. (Ames et al., 1995; Trichopoulos et al., 1996; Chauvenet et al., 2003).

Besides, the choice of lifestyle and habits such as tobacco, smoking and chewing, high alcohol intake, high fat diet and dietary habits, are also thought to initiate or promote various forms of cancers (IARC, 1993). About 40-60 percent of all cancers are related to our food choices and about one third of all cancer deaths may be related to what we eat. Besides diets high in fat, low in fiber, vitamins, fruits, vegetables, over weight and obesity, lack of physical activity etc have been associated with increased incidences of cancer (La Vechia et al., 1993; Rajkumar et al., 2003). Thus most of the prevalent human cancers to a significant extent can be prevented and many could be avoided by a suitable choice of lifestyle, diet and environment.

Carcinogenic risk from exposure to exogenous chemical carcinogens depend not only on the intrinsic nature and dose of each chemical, but also may depend on inter-individual variability in sensitivity to the carcinogens. An individual difference in the susceptibility to chemical carcinogens is one of the most important factors in the estimate of risk of human cancer (Clapper ML, 2000).

Oral cancer is sixth most common cancer worldwide and third most common cancer in developing countries accounting for about up to 40% of all cancers
(Parkin SM et al., 1988). Oral cancer includes cancer of lip, tongue, buccal mucosa, vestibule, gingiva, palate and floor of mouth and tongue being most common site for both males and females (Johnson NW et al., 1993; Park BZ et al., 1998). Oral cancer incidence increases with age and its rate varies widely throughout the world. India and South East Asian countries have highest rate of incidence of oral cancer while developed countries have low incidence rate of oral cancer. Incidence of oral cancer is increasing day by day due to more intake of various forms of tobacco and alcohol, which are considered to be the two most important etiological factors in the development of oral cancer. It is estimated that 75-90% of all head and neck cancers are caused due to the use of tobacco which includes cigarettes, pipes, cigars and smokeless tobacco (chew, dip, snuff, betel quid and areca nut). Tobacco users are between 20-40 times more likely to develop head and neck cancer than non consumers, depending upon the amount of use as well as the age, sex and race of the user (La Vechia C et al., 1997; Cawson RA et al., 1996; Graham S et al., 1997).

Tobacco smoke consists of more than thirty different carcinogenic compounds particularly nitrosamines and polycyclic aromatic hydrocarbons. Alcohol and tobacco act independently of one another to raise the relative risk of oral cancer (Elwood et al., 1984). In joint exposure risk, alcohol acts synergistically with tobacco (Notani et al., 1988; Sankaranarayanan et al., 1989, 1990). Oral cancer risk is related to these etiological factors by qualitative as well as quantitative point of view. It depends upon the duration of intake, age of onset, time since quitting, amount and type of tobacco and alcohol taken. Risk is also related to the demographic, cultural, socio-educational, occupational, dietary differences,
oral hygiene, compromised dentition and ionizing radiations (Notani 1988; Nandakumar et al., 1990; Hebert et al., 1993; Balaram et al., 2002).

Tobacco may be taken in various ways like smoking and chewing. Tobacco may be smoked in the form of manufactured cigarettes or indigenous forms like Bedi, Chutta (Agra), Chilum, Hooka (hubble-bubble) and pipe etc. There is strong dose-response relationship between tobacco intake and incidence of oral cancer. The most common form of tobacco chewing in India and Taiwan is betel quid. The 'Quid' for chewing consists of areca nut and pieces of unripe betel fruit or areca nut wrapped in a piece of betel leaf together with white or red lime. Betel quid chewing has a strong association with oral cancer which arises predominantly from surface epithelium with evolution from early premalignant lesions. The preneoplastic lesion may exist for years before invasion, and may behave persistently and progressively after abstinence from betel quid chewing. In Taiwan, there are about 2 million people who are suffering from the habit of betel quid chewing and approximately 80% of all oral cancer deaths are associated with this habit (Kuo et al., 1999).

The consumption of alcohol is linked to development of cancer in upper acrodigestive tract particularly the oral cavity and oropharynx. The exact mechanism is unclear but it is thought to be that due to a combination of local toxic effects on the mucosa and systemic effects from the associated dietary deficiency, hepatic damage and possible alteration in the patient's immunity. Infection agents have also been implicated as possible causes of cancer in the oral cavity like Treponema Pallidum, Human Papilloma Virus and Candida
albicans (Crispian Scully 1993). So there may be multiple etiological factors which play significant role in the development of oral cancer. In 1944, Willis stated that when carcinogenic stimuli affect epithelial tissue, all the epithelium in that area is affected similarly, but not necessarily equally. A neoplasm, therefore, is more likely to develop in tissue in which the stimuli have been maximal; however, similar neoplastic change may occur at a later stage in adjacent tissue that was exposed to the same carcinogen. The mucosa of the upper aero digestive tract should, therefore, be regarded as a field of growth that is constantly being bathed by the carcinogens and therefore will potentially have numerous areas of malignant and early malignant change. Early symptoms of oral cancer include persistent mouth ulcers (frequently painless), warty lumps and nodules, white red, speckled or pigmented lesions, recent onset of difficulty with speaking or swallowing and enlarged neck nodes. Any new oral lesion that persists longer than 3 weeks should be referred for a specialist opinion and also histopathological examination.

Incidences of oral cancer are very high in India and South East Asian countries in comparison to the western countries (IARC, 2002). Unlike in the west, most of the oral cancers seen in India, are preceded by distinct premalignant lesions such as leukoplakia and sub mucous fibrosis. The incidence of oral cancer is affected by age of the person, sex, site of cancer, religion, diet, tobacco and alcohol intake (Nandakumar et al., 1990; Balaram et al., 2002). Incidence of oral cancer increases with age and shows steep rise in the age group of 60 to 64. Oral cancer is more frequent in males than females with gender ratio between 2:1 to 6:1. The most usual site of incidence of oral cancer is tongue followed by floor of mouth.
Risk of oral cancer increases with increased use of alcohol and tobacco in its various forms. The effect of these factors have been studied by various researchers (Znaor et al., 2003; Jayant K. et al., 1987; Sankaranarayanan R. et al., 1990; Balaram P. et al., 2002). In this study, we analyzed the incidence of sex, age and site of cancer in oral cancer patients with tobacco and betel quid chewing habit in northern India. We also analyzed the relation of tobacco and betel quid chewing with oral cancer.

Oral carcinogenesis is a multi step process in which 6-10 genetic events lead to the disruption of the normal regulatory pathways that control basic cellular functions (Vogelstein and Kinzler, 1993). In recent years, several alterations in the expression of tumor suppressor genes and oncogenes in the development of oral squamous cell carcinoma (OSCC) have been described (Williams HK et al., 2000; Michalides. RJAM, 1999). Regular exposure of the oral cavity to betel quid and areca nut induces "field cancerization" involving initiation of different changes in the cellular DNA. Advances in the field of tumor suppressor genes and oncogenes have provided a tool to study the genetic changes occurring at different stages of carcinogenesis. In the present study, an attempt was made to investigate the expression of p53 and Cyclin D1 in tobacco and betel quid related oral SCC's in northern India.

The ability to metabolize carcinogens or pro-carcinogens, repair DNA damage, and control cell signaling and the cell cycle are fundamental to homeostasis. OSCC, under appropriate exposure to areca nut and betel quid, arise if these mechanisms are defective. Oral SCC arise as a consequence of multiple
molecular events induced by the effect of various carcinogens from habits such as tobacco and betel quid chewing, influenced by environmental factors, possibly viruses in some instances, against a background of inheritable resistance or susceptibility. Consequent genetic damage may affect many chromosomes and genes, and it is the accumulation of these changes that may lead to carcinoma in some instances, sometimes via a clinically evident pre-malignant or potentially malignant lesion (Field, 1992; Vokes et al., 1993). Although lifestyle factors play an important role in etiology, some patients appear susceptible because of inherited trait(s) in their ability or inability to metabolize carcinogens or pro-carcinogens, possibly along with an impaired ability to repair DNA damage. The characterization of genetic determinants for oral cancer susceptibility is important for understanding the disease pathogenesis and for defining preventive measures. There is growing evidence that a group of predisposing polymorphic genes exists, such as those involved in carcinogen metabolism, which may increase cancer incidence in certain environmentally exposed subjects, even when exposed only to low levels of carcinogens. Within preventive strategies, it is therefore necessary to identify these vulnerable members in our society, in particular those suffering from an unfavorable combination of high carcinogen exposure, cancer predisposing genes, and lack of protective (dietary) factors. Thus, molecular epidemiology faces the difficult task of analyzing carcinogen exposed individuals for a combination of "at risk" genotypes associated with higher cancer susceptibility. Rather than taking cancer as an end point, combinations of cancer predisposing genes can then be explored to better define gene environmental interactions and provide knowledge that should facilitate the identification of high risk subjects within carcinogen exposed population. Thus,
by identifying the "at risk" genotype combinations that may serve as markers, it is possible to identify susceptible consumers of tobacco and chewing products who are at risk for developing pre-cancerous lesions in a population based study.

In view of the rapid upsurge in the incidence of oral precancerous lesions, in both genders and high risk of transition to malignancy, there is urgent need to determine the relationship between polymorphisms in cancer susceptibility genes and risk of developing tobacco related oral precancerous lesions and cancers. The study will also help in understanding the mechanism underlying pathogenesis of the disease and aid in identifying markers for predicting high risk population.

An individual difference in the susceptibility to chemical carcinogens is one of the most important factors in estimate of risk of oral cancer. Most chemical carcinogens require metabolic activation by phase I enzymes (Cytochromes P-450) and detoxification by conjugation via the various phase II enzymes (epoxide hydrolase, glutathione S-transferase, N-acetyl transferase, sulfur transferase etc) (Ernster et al., 1991). Thus, the coordinate expression and regulation of phase I and phase II drug metabolizing enzymes and their metabolic balance may be an important host factor in determining whether exposure to carcinogen results in cancer or not (Idle et al., 1991). At present, it is accepted that most of the carcinogens in our environment are activated mainly by restricted number of P-450 species, including CYPIA1, CYPIA2, CYP2E1 and CYP3A (Guengerich et al., 1991; Kawajiri et al., 1991). CYP2E1 polymorphism.
has been associated with an increased risk for tobacco related diseases such as lung cancer.

Various cellular metabolic processes result in the formation of hydroxyl radicals that can cause oxidative damage to DNA (Demple B et al., 1994). This damage often results in single base changes that can be reversed by Base Excision Repair (BER) mechanism (Lindahl T et al., 1999). hOGGI and XRCCI are two of enzymes participating in the BER pathway, DNA repair system involved in the repair of damage resultant from oxidative stress. The enzyme hOGGI can recognize and excise OH-8-Gua, the major form of oxidative DNA damage induced by reactive free radicals (Burner S.D et al., 2000). XRCCI complexes with DNA polymerase via the NH2 terminus domain and with DNA ligase 111 via a blue ribbon commission on transportation (BRCT) domain to repair nicks or gaps left in the BER pathway (Nash R.A. et al., 1997). XRCCI has also been shown to be involved in the detection of single strand breaks between incision and ligation, and effect that likely occurs via poly (ADP-ribose) polymerase dependent and poly (ADP-ribose) polymerase-independent mechanisms (Masson M et al., 1998). Genetic polymorphisms of DNA repair genes have been reported to determine susceptibility to several cancers including lung, esophageal, bladder and nonmelanoma skin cancers (Perera FP, 1996). The aim of present study is to determine the frequency of polymorphism in DNA repair enzymes hOGG1, XRCCI and xenobiotic metabolizing enzyme CYP2E1 in relation to tobacco and betel quid chewing that may serve as markers to identify individuals susceptible to develop oral cancer.