Prologue

Oral Cancer scenario in India is very different from other parts of the world in terms of its etiology, sociology, magnitude, challenges, economy, psychology and other therapy related aspects.
Cancer is a multi-factorial, multifaceted and multi-mechanistic disease requiring a multi-dimensional approach for its diagnosis, treatment, and prevention. Worldwide, there were estimated 10.1 million new cases, 6.2 million deaths and 22.4 million persons living with cancer in the year 2000. The number will grow to 20 million new cases of cancer in 2020 with 12 million deaths (Parkin et al., 2001). In India, the annual estimates of cancer for the year 2001 was 0.98 million and the annual mortality in 2000 was 0.7 million (ICMR Bulletin, 2001). The incidence of cancer is on rise in India, with multiple risk factors that involve interplay between genetic and environmental components.

In certain countries some tumours seem to occur often and in other countries hardly at all. This can be explained in two ways. First, the high incidence of cancer may be due to an inherited predisposition to that tumour in the country of high incidence. An alternate explanation can be that the population in the high incidence country is exposed to carcinogens in their environment, which are not present in the country with a low tumour incidence. Epidemiological studies on the patterns of disease in various countries have shown remarkable differences in the incidence of individual tumours around the world. The majority of the differences are best explained by local environmental factors and not by an inherited tendency to cancer (Williams, 1985). For example, incidence of breast cancer in Japan is much lower than in western world. This difference in breast cancer is due to high dietary fat intake, obesity, and higher socio-economic status in Western countries. Incidence of colorectal cancer is high in North America, Western Europe, Australia/New Zealand and Southern South America as compared to Africa and Asia. These large geographic differences represent different environmental factors mainly dietary habits. Similarly, prostate cancer incidence is higher in USA than Japan, which reflects diet as main etiological factor (Parkin, 2001).
Oral cancer is the sixth most frequent cancer in the world accounting 3,00,000 new cases annually. The incidence of oral cancer is comparatively very low in Western countries, which is 2-6% of all malignancies. While in India, it constitutes nearly a third of all cancer cases. Annually, 75,000 – 80,000 new oral cancer cases are registered in India (National Cancer Registry Programme, 1996). Various cancer registries have documented that the high incidence is due to wide-spread habits of tobacco chewing and smoking (Gupta and Nandkumar, 1999) in Indian population. These differences in the incidence of oral cancer between Western countries and India may be due to difference in the habit of tobacco usage. Tobacco smoking (cigarette) is more prevalent in Western countries while tobacco chewing, smoking (bidi) and snuffing along with other ingredients like betel nut, Gutkha, lime, catechu etc. are the most prevalent habits in India (Jeng et al., 2001) (Figure-1). Due to these major etiological factors and increasing incidence rate it is suggested that oral cancer in India should be considered as a “new epidemic” (Gupta, 1999).

**Figure-1: Different forms of tobacco use in India and Western countries**
It has been known that free radicals produced during auto-oxidation of polyphenols in saliva of the tobacco users are crucial in the initiation and promotion of oral cancer (Jeng et al., 2001). Clinical, epidemiological and laboratory studies have confirmed an etiological relationship between prolonged tobacco chewing and oral cancer in India (Gupta et al., 1987, Jussawala and Despande, 1971). N-nitroso compounds constitute the most abundant carcinogens present in tobacco, with tobacco-specific nitrosamines (TSNA), representing an important class of genotoxic carcinogens (Hecht and Hoffman, 1988). Carcinogenicity bioassays have shown that N-nitrosonornicotine (NNN) and 4-(N-nitrosomethylamino)-1-(3-pyridyl)-1-butanone (NNK) are the most potent carcinogens amongst the TSNA (Hecht et al., 1983). The TSNA are metabolically activated to yield electrophiles, which react with cellular components, including nucleophilic centers of DNA leading to DNA damages (Loechler et al., 1984). Thus, there is continuous endogenous damage to cellular DNA by free radicals and accumulation of such damage has been found to play a significant role in carcinogenesis (Toyokuni et al., 1995) (Figure-2).

**Figure-2: Tobacco associated multi-step carcinogenesis in oral cancer**
In order to counteract these lethal effects, normal living cells have multiple antioxidant defense system in a cascade manner (Demple and Harrison, 1994). Non-enzymatic antioxidant vitamins such as vitamin A, E, and C have a number of biological pathways such as immune stimulation, inhibition of nitrosamine formation and alteration of metabolic activation of carcinogens. They can prevent genetic changes by inhibiting DNA damage induced by the free radicals. Vitamin E and C are reported to be significantly lower in cancer. While for oral cancer, it has been shown that administration of vitamin A and β-carotene can act as chemopreventive agents resulting into inhibition of progression of precancerous lesions into malignant transformation. Healthy smokers with long-term history of smoking have shown significantly elevated antioxidant enzymes such as superoxide dismutase and catalase while oxidative stress related changes showed decreased in glutathione levels and increased lipid peroxidation levels as compared to short-term healthy smokers and non-smokers. However, such kind of studies including effect of tobacco chewing in oral cancer patients are very few. Thus, it seems that studying biological markers, like antioxidant enzyme system and oxidative stress related markers could be helpful in early identification of tobacco associated oral cancer risk in healthy tobacco users as well as use in treatment monitoring of oral cancer patients.

Oral cancer cases in India frequently represent with local or regional metastasis at the time of diagnosis. The overall survival rate for patients with oral cancer is among the lowest (less than 50%) and has not changed during the past two decades (Nagler, 2002). Only 15% of the patients are diagnosed when the disease is at a localized stage. However, patients with advanced disease most often reflects the spread of the disease to local, regional and distant sites, events that are poorly controlled by combined surgery/irradiation.
A distinguished feature of malignant cells is their capacity to invade surrounding normal tissue and metastasize through the blood lymphatic system to distant organs. Experimental evidences have shown that cancer cells are armed with an array of proteolytic enzymes that are to be essential for the process of cancer dissemination. Tumour cell adhesion, deformability, motility and cell receptors also have important role in cancer metastasis. Invading cancer cells must breach barriers opposing their movement, which include basement membrane, stromal matrix and cell-cell junctions. The breaking of such barriers require involvement and interaction of many different types of cells, connective tissues, blood vessel components within different organs. Cell adhesion markers include E-cadherin while cell motility markers, which convert cell from static to a motile status, such as autocrine motility factor, autotaxin, and several growth factors have been used to study metastatic potential of tumour cells.

Matrix metalloproteinases (MMPs) particularly gelatinases (MMP-2 and MMP-9) have shown to play a central and early role in invasive and metastatic multi-step proteolytic events. (Curran and Murrau, 2000). Several studies on MMP-2 and MMP-9 activation in malignant oral cancer have shown elevated activation in malignant tissues as compared to normal tissues. MMP-2 levels are also found to be associated with lymph node metastasis. Therefore, studies on tumour markers addressing the mechanisms underlying invasiveness of oral cancer, like MMP-2 and MMP-9, may lead to early identification of metastatic potentials of oral cancer, which may consequently enable clinicians for better treatment alternatives and may improve quality of life and survival of oral cancer patients. There are no such reports from India and are remains to be explored.
Hence, the present study evaluated two aspects of oral cancer:

(1) Estimation of antioxidant enzyme activities and oxidative stress related markers in oral cancer patients and in tobacco habitual and non-habitual healthy population, as well as
(2) Activation of gelatinases i.e. MMP-2 and MMP-9, in oral cancer patients.

Accordingly this thesis is divided into two sections:

**Section-1:** Study of antioxidant enzymes and oxidative stress related markers in oral cancer.

**Section-2:** Study of MMP-2 and MMP-9 activation as markers of invasion and metastasis in oral cancer.