"There are many wonderful things but nothing is more wonderful than man"
- Sophocles.

Man unlike any other organism grows beyond his work, deduces concepts and eventually emerges ahead of his own accomplishment. The human system remains one of the most intriguing and fascinating subject in science. The essence of all scientific endeavors revolves around the well being of the human race (Steinback, 1939). As one explores phenomena or ideas at the frontiers of scientific knowledge, it is the unexpected that provides the clues to guide further work in research (Wolpert, 1995).

The presence of long-term risks associated with human exposure to mutagenic and carcinogenic agents has been revealed by classic epidemiologic studies. A number of progressive changes have occurred in workplaces, in the environment and in life-styles during the past few decades. Lifestyle factors are estimated to be responsible for about 80% of human neoplasias (Doll and Peto,
1981; Doll, 1990). Therefore, there is an increasing effort worldwide to determine the impact of environmental, genetic and life-style factors on genomic stability in human populations.

The use of Betel quid as a masticatory substance by human beings has been known since the 4th century A.D. in different parts of the world and is widely practiced in South and South-East Asian countries, Africa, as well as migrant communities from these areas to western countries. It is estimated that over 600 million people consume Areca nut in one form or another worldwide. Tobacco use in various forms always has been a major component of lifestyle factors. Betel quid alone or in combination with smokeless tobacco and/or smoking has been considered as major risk factors for oral cancer and pre-cancerous lesions in habituates (IARC 1885, 2004, 2007).

Oral cancer is an important health issue. It is the 6th most common cancer in the world. It accounts for about 2,63,000 new cases annually, two-thirds of which occur in developing countries (Parkin et al., 2003). The WHO predicts a continuing world wide increase in the number of patients with oral cancer, extending this trend well into the next several decades (Sciubba, 2001). The prevalence of the disease in different parts of the globe reflects different forms and extents of exposure to various etiological agents that include tobacco and betel quid. It is being associated with the occurrence of leukoplakia, erythroplakia and oral submucousal fibrosis and also believed to be associated
with oral cancer. In India, the age standardized incidence rates (ASR) of oral
cancer per 100,000 populations are 12.8 in men and 7.5 in women (Ferlay et
al., 2001). Collaborative data using reports from the National Cancer Institute
(NCI), Indian Council of Medical Research (ICMR) show alarming picture of oral
cancer incidence.

Betel quid consist of the leaf of piper betel wrapped around Areca nut,
lime, with or without tobacco along with additional condiments like cardamom,
cloves, catechu extracts. It has been reported that Areca nut users, independent of
tobacco use, are 100 times at higher risk to get oral submucous fibrosis (Maher
et al., 1994). People with oral submucous fibrosis were 19.1 times more likely to
develop oral cancer than those without it, after adjusting for smoking, and for
use of alcohol, naswar, and paan with or without tobacco (Merchant et al., 2000).
Moreover, betel quid may act by its ability to physically destroy mucosal
membranes, thereby establishing gateways for viral infection.

Although epidemiological studies show that high incidence of oral cancer
are associated with betel quid chewing in Oriental countries, these data require
detailed investigation because food habits, genetic susceptibility and betel quid
chewing practices vary considerably among different countries and even in
different localities within the same country. Studies on ethnic differences and
prevalence of oral cancer and precancerous lesions are scarce. Epidemiological
information provides vital clue to plan and evaluate strategies against a disease.
Primary prevention has been widely advocated as the optimal strategy for the control of oral cancer in the long run.

Health suffers the influence of inherited, nutritional, and environmental factors. Populations of industrial areas are intensely exposed to chemical substances that can cause mutations, cancer, and congenital defects (Hirvonen, 1995). Exposure to genotoxic agents can induce several types of cancer, such as urinary tract, skin, larynx, and pancreas cancers, and leukemias (Santos-Mello and Cavalcante, 1992). It is necessary to identify the exposure to hazardous agents in order to devise suitable measures to decrease their presence in the environment or to protect the population against them can minimize the consequences on people's health. One way to study the effects on an exposed population is to conduct monitoring studies, using pertinent biological parameters with a short-term manifestation, such as cytogenetic analysis, by which damages to the DNA or to the chromosomes resulting from exposure can be identified. The obtained information can be used as an early warning about the potential risk of health problems developing in the long run (Au, 1991).

Because it has never been acceptable to study toxic substances in humans, investigators have used animals and in vitro systems to predict human risk to set safe drug dosages, to regulate levels of environmental exposure and even to determine whether a substance should be used at all. This approach has limitations as we cannot always be sure that the results obtained with fungi,
bacteria or even animal trials apply to humans (Garattini 1983; Efron 1983). However, one technique that has gained wide popularity is the measurement of micronuclei (MN) in peripheral blood lymphocytes, epithelial cells, erythrocytes, and fibroblasts of human beings because of its non-invasive nature. With better understanding of the multistage carcinogenic process, there appears to be a window of opportunity for genetic monitoring using cytogenetic endpoints in suspected high risk populations. For many years, blood has been seen as the ideal matrix for human biomonitoring studies as it is in contact with all tissues and in equilibrium with organs and tissues. However, blood sampling is an invasive procedure and suffers from ethical and practical constraints. Therefore, the use of non-invasively collected matrices for human biomonitoring should be promoted as an ethically appropriate, cost-efficient and toxicologically relevant alternative for many biomarkers that are currently determined in invasively collected matrices (Smolders, 2009).

Micronuclei provide a measure of both chromosome breakage and chromosome loss and it has been shown to be at least as sensitive an indicator of chromosome damage as classical metaphase chromosome analysis. The key advantage of the MN assay is the relative ease of scoring and the statistical power obtained from scoring larger numbers of cells than are typically used for metaphase analysis (Fenech, 1999). Exfoliated cells hold strong potential as a tool for biomonitoring human populations exposed to genotoxic agents or undergoing preventive treatments. In many cases, epithelial tissues are the
actual targets of carcinogens, as indicated by the sites of cancers related to the exposures. Epithelial tissues are in immediate contact with inhaled and ingested genotoxic agents, kidney and bladder cells are also in contact with metabolites of the chemicals; genotoxic changes in bronchial, esophageal, cervical, breast duct and other types of epithelia have been reported. More than 90% of cancers arise in epithelial tissues (Doll and Peto, 1994); in many cases, these tissues are the actual targets of carcinogens, as indicated by the sites of cancers related to the exposures. Epithelial cells can be easily collected from the mouth, nose, and bladder by noninvasive procedures. The standard laboratory procedure is feasible, cheap and accurate; final results can be obtained in hours.

Micronuclei study has been widely acknowledged as an effective biomarker in genotoxic studies and a series of studies conducted in different laboratories across the world suggest the association of the frequency of MN in target or surrogate tissues and cancer development. The assay was developed in the early 1970s and was first used on human blood cells by a Canadian researcher at York University, in Toronto (Heddle 1973). Dr. Hans F. Stich, head of the BC centre's Environmental Carcinogenesis Unit, and his colleague Dr. Miriam P. Rosin have subsequently been instrumental in pioneering the test's further application to exfoliated cells (Lee, 1985). A significant increase of the MN frequency in target tissues as well as in peripheral lymphocytes in cancer patients has been reported (Duffaud, 1997). Subjects affected by certain congenital and dementia diseases (Bloom syndrome, ataxia telangiectasia,
Alzheimer’s disease, Down’s syndrome) have both abnormally high MN frequencies and an increased risk of cancer (Rosin, 1985; Thomas, 2007, 2008). Certain clinical chemoprevention trials on oral premalignancies have used MN in oral mucosa as surrogate endpoint of cancer (Benner, 1994; Desai, 1996). There is a strong correlation between carcinogenicity and genotoxicity for some agents able to increase MN frequencies in humans and animals like ionizing radiation, ethylene oxide, benzene, tobacco smoke (Sorsa, 1992). Micronucleus frequency is associated with the blood concentration of vitamins and folates, which are positively correlated with increased risks for some cancers (Blount, 1997; Fenech, 1997; Davis, 2003). All these and similar other studies have successfully transformed a hypothesis into certitude: micronuclei in epithelial exfoliated cells represent a trustful diagnose element, and have a great potential in being considered and used as biomarker in chronic exposures to genotoxic hazards (Havårneanu, 2004). The HumanXl Project further establishes the significance of MN study in epithelial tissues (Bonassi, 2009).

Addiction to tobacco and betel quid is a conscience exposure to harmful chemicals and their interactive products apart from other man made chemicals and their mixtures present in the environment. Tobacco use in various forms always has been a major component of life style factors. Therefore, it is important to assess the effects of newer forms of tobacco preparations like sadagura alone or in combination with other such habits. The highest incidence of oral
cancer in India is reported in Assam in the North-East region, where it is the second leading cancer among men and third among women (Phukan 2001).

Betel quid alone or in combination with smokeless tobacco and/or smoking has been considered as major risk factors for oral cancer and precancerous lesions in habituates (IARC, 1985, 2004, 2007). In India, smokeless tobacco in the form of gudakhu, mishri, snuff, chewing tobacco, masheri and mawa are consumed with or without Areca nut (Gupta, 1984). However, in the southern part of the state of Assam and the adjoining areas of the states of Meghalaya and Mizoram, a very unique preparation of smokeless tobacco known as ‘sadagura’ is highly popular among the local population. Sadagura is a smokeless tobacco preparation, contains sun dried and roasted tobacco leaves along with very small amount of fenugreek seed and aniseed for flavor. It is chewed and swallowed mostly with betel quid unlike most other smokeless tobacco preparations, which are rubbed on the teeth and gum. The stimulating effect coupled with strong flavors results in addiction among the users who consume sadagura without being aware of its ill effects. Further, this becomes more relevant especially because of the fact that this valley registers a very high frequency of oral cancer compared to other parts of the country and no such study to stratify susceptibility to oral cancer and precancerous lesion in the region among betel quid chewers has so far been done. In the light of the above discussion, the proposed study aims at addressing the following objectives.
Objectives:

In view of the above considerations, the major objectives of the present study are:

1. To establish the normal baseline frequencies of DNA damage in the study population in terms of micronucleus frequency in buccal epithelial cells.

2. To determine micronucleus frequency in individuals with different chewing habit and compare it with the control population.

3. To determine micronucleus frequencies in individuals with different kind of tobacco uses habits and compare it with the control population.

4. To find correlation between micronucleus frequency and oral precancerous lesions and oral cancer incidence.

5. To determine the main demographic and environmental variables those influence the micronucleus index in the study population if any.