The current study was carried out with the following major objectives:

1. To establish the normal base-line 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.
In this study emphasis was given to exfoliated and basal cells of the buccal cavity, having various nuclear damage (Cytome Assay) among the chewers, persons with precancerous and cancerous lesions and individuals from different occupational setup. Micronucleus being a very important indicator of genetic instability, the total baseline micronucleus frequency of chewers and non-chewers was also compared. The buccal cytome assay involves cellular/nuclear classification specific for DNA damage, potential cell proliferation and cell death markers. The participants were divided into groups without any chewing habit, habituates with chewing of betel quid with or without tobacco, tobacco smokers and multiple habit users as well as subjects with oral leukoplakia, erythroplakia, erythroleukoplakia and oral submucous fibrosis to determine changes related to malignancy.

The percentage of baseline micronucleus frequency in the non-chewing group was found to be 0.15 (0.09 for males and 0.20 for females). A variation of this value was observed among certain individuals and any etiological factor could not be assigned to them and this may be due to individual genetic susceptibility.

There was significant increase in cells with micronucleus and nuclear bud in habituates irrespective of their chewing habit (P<0.001) indicating DNA damage and a positive role played by tobacco. Binucleated cells were significantly increased in all the groups and were higher in habituates who chew betel quid
as a component of chewing. It is possible that the alkaloids present in the betel nut may be responsible for either inducing nuclear division or block the pathways leading to cytokinesis and thus cells cannot separate following nuclear division. The percentage of cells with karyorrhexis, karyolysis, pyknosis and condensed chromatin was also elevated in all the habituate groups these are cell death markers and may be induced by either betel nut alkaloids or tobacco alkaloids or their metabolites.

The percentage of cells with micronucleus and nuclear bud was significantly elevated in all samples from precancerous lesions when compared to the control (P < 0.001). There was also a significant increase in basal cells and binucleated cells (P < 0.001) in these groups. The percentage of cells with karyorrhexis, karyolysis, pyknosis and condensed chromatin was also significantly elevated in all these samples from precancerous lesions (P < 0.001). Among all the parameters, the percentage of condensed chromatin cells was highest in buccal cells from individuals having precancerous lesion. This is followed by karyolytic cells. However, the incidence of pyknotic cells was highest in oral submucous fibrosis indicating a different mechanism involved in oral submucous fibrosis. However a detail study is required to confirm this observation.

The percentage of cells with micronucleus and nuclear bud was significantly elevated in subjects with oral squamous cell carcinoma when compared to the
control group. (P< 0.001). There was a significant increase in basal cells and binucleated cells (P< 0.001). The percentage of cells with karyorrhexis, karyolysis, pyknosis and condensed chromatin was also significantly elevated in all the patient groups (P< 0.001). When we evaluated all the markers in the cytome assay we found that the incidence of karyolytic cells was highest in the cytome profile in buccal epithelial cells of subjects with oral squamous cell carcinoma followed by binucleated cells.

There was a significant increase in cells with micronucleus and nuclear bud in tea plantation workers involved in tea plucking, tea dusting and farmers (P< 0.001). Binucleated cells were also significantly increased in both the groups (P< 0.001) and was higher in tea plantation workers. The percentage of cells with karyorrhexis, karyolysis, pyknosis and condensed chromatin was also elevated in both the groups at different levels of significance. Most of the subjects had the habit of chewing paan with smokeless tobacco, smokeless tobacco, smoking tobacco or were multiple habit users. It was interesting to note that the level of damage was much lower in farmers who did not have any chewing or smoking habit which further confirms the role of betel quid and tobacco in causing genotoxic and cytotoxic damage in the study group.

We developed and standardized the changes in the nuclear shape in buccal epithelial cells in our study. Different types of nuclear shape was recorded during the course of study. It was observed that there was a correlation
between DNA damage and cytotoxicity parameter and nuclear shape abnormalities in all the groups. The damage level was higher in precancerous conditions and maximum in oral squamous cell carcinoma. Although the mechanism involved in such changes in nuclear shape abnormalities could not be evaluated, it appears that some pathways leading to cytotoxicity, genotoxicity, may play a role in induction of such changes. Hence, the nuclear shape abnormality can be considered as an additional parameter to study changes in buccal epithelial changes and to gauge the risk of cancer susceptibility.

It is evident that chewing of paan with or without smokeless tobacco, chewing tobacco alone, smoking tobacco cause genotoxic and cytotoxic damage. Damage level was elevated when multiple habits were practiced by the same individual probably suggesting a synergistic effect. The chewing habit which had been found to be widely practiced by the majority of the population in Cachar district appeared as the probable major etiological factor for the high incidence of oral squamous cell carcinoma reported from this region.

These changes show distinct differences between the cytome profile of normal individuals without any chewing habit relative to that for a precancerous and malignant conditions and highlights the diagnostic value of the cytome approach for measuring changes in genome damage, cell death and cell proliferative effects. It is important to verify these results in a larger group to evaluate its clinical usage. Future studies are important to determine if these
changes are specific to malignant conditions or whether they reflect alterations in the cellular kinetics of the buccal mucosa that can be linked to non-specific pathological situations also. Thus, the same parameters need to be measured in mucosal cells to screen high-risk groups in general population. Furthermore, it is important to determine whether subjects who have different genetic forms of familial susceptibility exhibit the same changes and whether these changes can be identified presymptomatically. Further improvements in methodology involving image analysis and flow cytometry should be investigated and subsequently validated, to allow the development of an automated system that could potentially allow for scoring larger numbers of cells, in order to more accurately define changes in cellular kinetics and genomic instability events within the buccal mucosa.

In conclusion, changes within the buccal micronucleus cytome assay may be useful as potential biomarkers to gauge DNA damage and in the identification of individuals associated with premalignancy and malignancy. In the future the buccal cytome may form the basis of a noninvasive diagnostic test that may eventually be used to identify presymptomatically those individuals with an increase risk of developing oral squamous cell carcinoma. The nuclear shape abnormality seemed to be a promising endpoint to be included in future studies. It may then be possible to develop and implement potential prophylactic measures based on dietary intervention studies that may result in a cessation or reversal of the changes that are characteristic of the
disease. Buccal cytome changes may eventually be used to reflect disease severity or as a within subject biomarker to gauge the effectiveness of preventative interventions aimed at slowing down the progression of the disease.

The buccal cytome assay combined with the study of nuclear shape abnormality therefore hold great promise as a future research tool and can be applicable to lower the incidence of cancer burden.