Summary
And
Conclusion
In the present study, both target tissues i.e. buccal mucosa cells and non-target i.e. peripheral blood cells were used to assess the cytogenetic alterations among chewers. A cross sectional study was conducted to find out the association between oral health status with respect to chewing habits, the genotoxic potential of these substances by studying the buccal and blood cytome assay, CA assay, comet assay, interphase FISH of p53 gene and cotinine, copper and zinc level among chewers and non-chewers.

The subjects were enrolled randomly and divided according to their chewing habits as chewers (120 subjects) and non-chewers (99 subjects). They were further characterized on the basis of their chewing habits as areca nut with tobacco (85 subjects), areca nut without tobacco (15 subjects) and only tobacco chewers (20 subjects). A detailed history was recorded on predesigned proforma. Clinical examination of oral cavity was performed. Interincisor and intermolar distances were also measured to note the role of chewing habit on mouth opening. Micronucleus as well as other nuclear anomalies were studied in the buccal mucosa cells as well as blood cultured cells of chewers and non-chewers. Chromosomal aberration, DNA damage by comet assay and FISH p53 gene were assessed in blood cells of chewers, OSMF subjects and non-chewers. Plasma cotinine level was measured using HPLC. Copper and zinc levels in serum were measured in representative number of samples by atomic absorption spectrophotometer.

The data on subject and demographic characteristics revealed that about 93.33% of chewers were male as compared to 6.67% female. More
number of chewers were found in lower educational level i.e. up to secondary level. However, the number of subjects with higher education were more among non-chewers with respect to chewers indicating the role of education in refraining from the chewing of tobacco and areca nut. Two-third of chewers were in the age group of 20-39 years. Data also revealed that about 3.3% of chewers were in the younger age group (up to 19 years). Further analysis indicated that about two third (66.6%) of the chewers have started chewing before the thirty years of age. About 30% of the chewers started chewing even before the age of 20 years indicating the role of age in the initiation of chewing habit. Most common cause for initiation of chewing habit was found to be encouragement by friends (peer pressure). Other reasons cited for initiation of the habit included self-pleasure. This suggests that chewers acquired the habit of chewing tobacco and areca nut at early ages and peer pressure might also be an important factor for initiation of the chewing habits.

Occupational distribution of the subjects revealed that physical laborers (61.67%) were more among chewers. Both chewers (94%) and non-chewers (96%) were aware that chewing could cause cancer. Further, higher number of non-chewers (51.51%) were aware that chewing habit could drain economy as compared to chewers (38.33%). However, most of the chewers considered chewing as a bad habit and were willing to quit the habit. The data revealed that higher number of chewers i.e. 40.83% were consuming the quids up to 4 times a day, whereas 37.5% were using the quids 5-8 times/day. Maximum number of the subjects i.e. 30.8% were having the habit of chewing for < 4 years and equal number (24.17%) of the subjects having the chewing habits for the last 5-8 and 9-12 years.

Prevalence of smoking habit (past and present) was about 26% and 17% among chewers and non-chewers respectively. The data indicated that the habit of alcoholism was significantly higher among chewers as compared to non-chewers. The data on oral hygiene status indicated that non-chewers had significantly good oral hygiene status as compared to chewers. Oral mucosal lesions were higher among the chewers than non-chewers. All the
OSMF subjects were chewers indicating the role of chewing in causation of OSMF. Other lesions included leukoplakia (6.66%) among chewers as compared to (1.01%) non-chewer (having the habit of smoking). These data suggests the role of chewing mixtures in causation of oral soft tissues lesions. The mean distances between interincisor and intermolar were significantly lower in the chewers and OSMF subjects indicating the role of chewing in restriction of mouth opening.

Buccal nuclear anomalies were observed to be elevated among the chewers and OSMF subjects with respect to non-chewers. Chewing has significant positive effect on the levels of MN, N buds, bi-nucleus and karyorrhexis whereas negative effect on the level of normal cell differentiation. However, MN frequency as well as N buds, binucleated cells and karyorrhexis among areca nut with tobacco chewers found to be increased as compared to non-chewers. Further, elevation of nuclear anomalies was found in the subjects with higher chewing frequency in quids/day and duration in years. Significant positive correlation was found between the genotoxic biomarkers MN and N Buds. A significant negative correlation was found between normal differentiated cells and cell proliferation markers - binucleated cells. Similar results were obtained with cell death parameters - karyorrhexis cells and karyolitic cells. This clearly indicates towards the direct genotoxic potential of the areca nut and tobacco chewing. The frequency of buccal MN, BN MN and karyolysis cells correlated positively with LCE.

Increased percentage frequency of nuclear anomalies was found in binucleated cells of areca nut and tobacco chewers and OSMF subjects as compared to non-chewers using CBMN assay. Chewing has significant positive effect on the levels of BN MN, total MN and NPB. Further, the data revealed that average percentile of BN MN among chewers and OSMF subjects were significantly higher as compared to non-chewers. Nuclear buds and apoptosis were increased among chewers and OSMF subjects. Average percentile of BN MN, total MN and NPB in areca nut with tobacco was significantly higher as compared to non-chewers. A significant positive
correlation was found between the blood cytome parameter of BN MN with multi MN, NPB and total MN. Significant positive correlation was observed between apoptotic cells and DNA misrepair marker i.e., NPB. The frequency of BN MN, NPB and N buds correlated positively with LCE.

Chromosomal aberration was significantly increased in chewers, among chewers of areca nut with tobacco and OSMF subjects as compared to non-chewer subjects. Significant elevation was also found in the frequency of chromosomal aberration/cells and chromatid gap associated with consumption (chewing frequency/day) and duration (years) of chewing habit. The frequency of chromatid gap, chromatid break and chromosomal aberration per cells correlated positively with LCE. However, the frequency of normal metaphase cells correlated negatively with LCE.

There were significant differences in % tail DNA using comet assay between non-chewers and chewers and also in the areca nut with tobacco chewing group. The tail moment and OTM were significantly higher in chewers and only tobacco chewers. Further, OTM increased significantly in areca nut with tobacco chewers group as compared to non-chewers. In addition, OSMF subjects also showed increased % tail DNA, tail moment and OTM as compared to non-chewers. DNA damage was found to be increased in the subjects with higher chewing frequency/day and duration of chewing in years with respect to non-chewers.

Plasma cotinine level was detected in 88.75% and 54.45% of the chewers and non-chewers respectively. The mean cotinine level was found to be 78.98 ng/ml and 59.04 ng/ml among chewers and non-chewers respectively. However, 11.25% and 45.55% of the chewers and non-chewers showed cotinine level below the detection limit. The detection among non-chewers might have resulted from the environmental tobacco smoke (ETS). A positive linear correlation was observed between the lifetime chewing exposure and the plasma cotinine levels.
Analysis of trace elements revealed that serum copper level was highest in chewers and subjects with OSMF followed by non-chewers in descending fashion. Serum zinc level was found to be lower in chewers and subjects with OSMF as compared to non-chewers, though the difference in each group being statistically non-significant. Copper level positively correlated while zinc level negatively correlated with the frequency of chewing quids per day, but these changes were statistically non-significant.

Assessment of chromosome 17 and p53 gene has been performed by dual colour FISH to detect structural and chromosomal abnormalities. The data indicated significant increased frequency of 17cen–p53 deletions in chewers and chewers with OSMF subjects as compared to non-chewers. Similarly, the chewers and OSMF subjects showed a statistically significant (p<0.001) increment of 17p gain. Elevation has been observed in the percentage of p53 deletion and p53 gain in the subjects with higher chewing frequency (quids/day). Similar results were obtained with the duration of chewing in years. Positive correlation (significant) was found between the p53 gene deletion and gain with duration and frequency of chewing.

In the present study, a positive correlation in MN was observed between target (Buccal MN) and peripheral (Blood MN) tissue. Significant positive correlation was also observed between buccal MN and chromosomal aberration per cells. OTM by comet assay showed positive correlation with MN in binucleated cells and CA/cells. The deletion p53 gene has been observed to be positively correlated with olive tail moment and apoptosis in buccal cells. Further, apoptosis in buccal mucosa cells positively correlated with olive tail moment whereas micronucleus in buccal cells was observed to be correlated positively with serum copper levels.

In conclusion, the study clearly indicates that chewing of areca nut and tobacco have adverse effects on oral mucosa as evident through the higher number of cases with precancerous lesions like OSMF among chewers. This study has also identified the areca nut and tobacco associated genetic and
non-genetic cell changes, which have been assessed using different assays. Further, some of the assays has been identified as useful biomarkers in cytogenetic investigations and can be used for screening of areca nut and tobacco chewers for the early detection of oral lesions. The increased frequency of micronucleus and chromosomal damage indicates the genotoxic and cytotoxic potential of areca nut and tobacco. These results provided the valuable data in order to better understand the genotoxic potential of areca nut and tobacco, as well as to assess the biological risk to OSMF subjects.

Since, areca nut and tobacco chewing is the most prevalent habit in South east Asian countries and also oral cancer in this region, therefore it is necessary to adopt Information Education Communication (IEC) about the ill effects of tobacco and areca nut in the community which will help in the prevention of oral cancer.