Discussion
The epidemic of chewing areca nut with or without tobacco is one of the greatest threats to global health today. It is estimated that at least 600 million individuals consume areca nut in one form or another worldwide (Warnakulasuriya and Peters, 2002). The habit is widespread in Southeast Asia and the South Pacific and in people of Indian origin migrated to elsewhere in the world. The betel quid chewing habit is in fact found all over the world wherever Indians have settled. In developing countries especially in South East Asian countries, there has been a sharp rise in this habit especially amongst the youth and the tobacco industry continues to target them. Young children start using sweetened areca nut products, often adding tobacco later in their adolescence. In addition a new habit i.e. panmasala with or without tobacco chewing is increasing rapidly in Indian subcontinent and is being consumed even by those who generally refrain from smoking and tobacco chewing. Increasing use of tobacco and betel nut chewing habits especially panmasala with or without tobacco even by the vulnerable group of the society i.e. children and pregnant women and available data on the role of different chewing habits and cancer, it is suggested to look in to the hazardous effect of those habits to the society (Kumar and Saiyad, 1999). Further, as smoking is banned in public places in most of the countries, more and more people are switching over to smokeless tobacco chewing products. Thus there is an urgent need to address this important issue by conducting research and also impart education.
Approximately one-third of the adult population in the world uses tobacco in some form and half of them dies prematurely. According to an estimate by the World Health Organization, 4.9 million people worldwide died in 2000 as a result of their addiction to nicotine (WHO, 2002). This huge death toll is rising rapidly, especially in low- and middle-income countries where most of the world’s 1.2 billion tobacco users live. Developing countries also account for about half of the world’s disease burden related to tobacco as measured by DALY i.e. Disability Adjusted Life Years (WHO, 2002). Within these countries the prevalence of chewing habit is highest amongst people of low educational background and among the poor and marginalized. This segment of the population is already affected by malnutrition and infections.

The shift in the global pattern of chewing areca nut and tobacco habits is reflected in the changing burden of disease pattern and related deaths. Sadly, the future appears worse. Because of the long time lapse between the onset of habit and the inevitable wave of disease and deaths that follow, the full effect of today’s globalization of marketing and increasing rates of usage in the developing world will be felt for decades to come. Even today, about one-third of the total cancer cases are related to oral cavity in the Indian subcontinent. The higher percentage of oral cancer is believed to be associated with chewing and smoking habits. Chewing and smoking are a major preventable cause of premature death and also a common risk factor to several general chronic and oral diseases. Chewing habits are widely prevalent in a number of countries of the world depending on socio-cultural conditions. The habit may include areca nut, tobacco, betel nut, pan masala or a combination of these. Pure areca nut contains tannins of which gallo-tannic acid and D-Catechol are important. It contains several alkaloids of which arecoline is most abundant: arecaidine, guavacoline and guavacine are also present in small quantities. Other substances comprise water, carbohydrates, proteins and mineral matter (Murti et al. 1994).
There are several types of chewing habits in India featuring use of betel quid (fresh betel leaf, fresh areca nut, slaked lime, catechu and tobacco), pan masala (plain pan masala-areca nut, slaked lime, catechu, condiments and with tobacco-gutkha), mainpuri (tobacco, slaked lime, areca nut, camphor and cloves), mawa (areca nut, tobacco and slaked lime), khaini (tobacco and slaked lime), and other smokeless tobacco preparations (mishri, gudhaku, bajjar etc.). They can also contain other sweeteners and flavoring agents. There is some confusion in the reporting of “betel quid” and tobacco chewing habits. At a recent workshop in Kuala Lumpur it was recommended that “quid” be defined as “a substance, or mixture of substances, placed in the mouth or chewed and remaining in contact with the mucosa, usually containing one or both of the two basic ingredients, tobacco and/or areca nut, in raw or any manufactured or processed form” (Chiba, 2001).

Areca nut usage with or without tobacco is a primary cause of many oral diseases and adverse oral conditions. Most oral consequences of tobacco use impair quality of life be they as simple as halitosis, as complex as birth defects, as common as periodontal disease or as troublesome as complications during healing. Tobacco-induced oral diseases contribute significantly to the global oral disease burden. In comparison to smoking habits, the patterns of chewing habits and their influence on health of chewers are less documented. The reason is obvious. The habit is encountered predominantly in the under developed or developing countries and developed nations paid least attention on this important problem. Thus, the present study was conducted to study the role of various chewing habits, especially areca nut, tobacco and commercial products such as pan masala on soft and hard tissue status of oral cavity; the genotoxic potential of these substances by observing micronuclei in the buccal mucosal cells; copper and zinc levels in the saliva and serum; immunoglobulin levels in the serum samples of representative number of chewers and non-chewers etc.
In this study, 365 apparently healthy patients were enrolled randomly and divided as chewers (168 patients) and non-chewers (197 patients) for comparison. The subject characteristics and demographic data revealed that most of the subjects were from the urban area in both the groups. This could be explained as the study was conducted at the hospital situated in the urban area. The data further suggested that most of the chewers as well as non-chewers were from the Hindu community followed by Muslims reflecting the population structure of this part of the country. The population structure data of Gujarat indicated that majority of people belong to Hindu community followed by Muslims.

The present studies revealed that majority of chewers were young (aged 20-39 years). When the distribution of chewing habits is examined, it is interesting to note that there is a significant increase in the incidence of habit with age up to 30 years. Further, most of the subjects (about 78.5%) reported age of initiation of chewing habit between 10-29 years. Two subjects started the chewing even before the age of 9 years. This indicates that the chewing habit starts at an early ages. This might be due to curiosity, mass media advertisement to attract the youth and sometimes-prevailing social customs or habit prevalent in the family. Chandra and Ganguli (2002) also reported that smoking and chewing habits are formed in the age group of 15-25 years. Thus, there is a need to educate the youth about the adverse health effects of tobacco and areca nut chewing and to prevent them from indulging in habits. Habit is more prevalent in age group of 10-29 years, and thereafter, this habit tapers off gradually. This might in part be due to the eventual loss of individual's teeth, which prevents him from indulging in the habit anymore.

Males comprised almost 95% of chewers' group in the present study. Higher prevalence in males is in confirmation with those of earlier studies of Pindborg et al. (1967), Yunus and Khan (1997) and Chandra and Ganguli (2002). Further, lower number of females in the present study might be due to
the fact that they are generally reluctant to participate in such type of study. There are clearly many differences in the way areca nut is consumed-on its own or often in combination with many other ingredients, including tobacco. In India, there are 38 different combinations of areca nut and tobacco use reported by Pindborg et al. (1960). The distribution of habits in the present study revealed that various forms of quids included gutkha, mawa, khaini, pan, pan masala etc. but majority of the chewers included tobacco in some form in the quid. Earlier studies have also pointed out that, in India, most of habitual betel quid chewers include tobacco in the quid. For example, in studies of 1,50,000 people in different areas of India, 34,000 were chewers and only 3% did not include tobacco in the quid (Mehta et al. 1971, 1972). Our study also indicates that 86.91% chewers were using tobacco as one of the components as compared to 8.33% chewers without tobacco. Rest of the 4.76% chewers had mixed chewing habits i.e. using both tobacco and without tobacco chewing products.

In the present study, most of the chewers were educated. With the increase in educational level the prevalence rate was noted to go up, with most of chewers having studied up to primary or secondary level. This carries significance as this educated section of the society, which constitutes the major chunk, may be conveniently targeted for eradication of habit through mass media, awareness and educational programmes.

Most of the chewers in the study were physical labourers followed by industrial workers, office workers, and students in decreasing order of frequency. Waerhaug (1967) quoted that the habit of betel consumption is particularly prevalent among the lower classes. The higher number of chewers in physical labourer group might be due to the fact that they use these products for some sort of feeling relaxation and also due to addiction for nicotine.
Ko et al. (1992) studied the prevalence of betel quid chewing habit in Taiwan in a group of Chinese people from Kaohsiung city and in a second group from the aboriginal inhabitants of South Taiwan. They noted that lesser educated older men, blue collar workers, smokers and drinkers were most likely to be betel chewers. Further, it has also been reported that increasing number of areca nut users are found among white-collar workers, especially officers and students, while the number among blue-collar workers, though still higher than the white-collar workers, is declining (Lu et al. 1993). In the present study, considerably higher number of subjects belonged to group of office going workers and students. Chaturvedi et al. (1998) reported that age and occupation had significant association with tobacco use but the influence of educational status on chewing habit was low. A significant association of chewing habit with the physical labourers was also observed in our study.

The present study revealed that there was significantly higher prevalence of chewing habit in family of chewers than non-chewers. About 42.3% chewers reported the chewing habit in family as compared to only 29.4% in non-chewers family. This indicates that family environment has a definite role in acquiring chewing habit. Lu et al. (1993) also stated that the example of the father or other male family figures had some effect on acceptance of chewing habit by the children. It has been suggested that children living in a home with parent with drinking habit are more likely to experience the same due to the "intergenerational effects". However, the mechanism of intergenerational transmission is still obscure. Although not statistically significant, incidence of cancer was also higher in family of chewers than non-chewers. Though not directly, but this may be correlated with higher prevalence of chewing in family of chewers. Further, most of the chewers as well as non-chewers were aware that chewing habit might lead to cancer. Also, significantly greater number of chewers felt that chewing was a bad habit, drained economy and most of them were willing to quit the habit. Hence, necessary efforts should be made to stop the youth for indulging in
the habit through information, education and communication about the ill
effects of the chewing habit. This can be successfully achieved only through
aggressive prevention programme through mass media.

Analysis of the distribution of subjects according to site of placement
revealed another interesting finding. Whereas chewers chewing quid without
tobacco generally did not place the quid constantly at one place (except one
subject in the entire study who placed it against buccal left mucosa), about
50% of chewers using product with tobacco preferred to keep the quid at one
fixed place in the oral cavity constantly. This practice might also be
responsible for the higher prevalence of oral disease especially oral cancer at
the site of placement. Earlier Hirayama (1966) reported that the site of origin
of oral cancer corresponding to the area of placement of tobacco quid. Later
Goud et al. (1990) also reported that oral cancer occurs almost always on the
site of the mouth where tobacco quid (particularly night quid) is kept. Thus
keeping the quid at one place constantly also had additional risk factors for
oral cancer.

Encouragement by friends (peer pressure) rated highest among
causes of initiation of chewing habit. Other cited reasons by the chewers for
chewing betel nut included curiosity, it causes euphoria, increases salivation,
satisfies hunger, relieves tooth pain and ameliorates nausea in pregnant
women. Chewing and smoking behavior and related attitudes of peers, family,
and media could be expected to affect the adolescent's attitudes, beliefs,
values, expectations, and learned behaviors (Wang, 2003). Young
adolescents often perceive smoking and chewing as glamorous or as a
behavior distinctive to adults. Development of habit is a complex
phenomenon involving several intrapersonal and environmental factors. It is
hypothesized that both social environmental and personality determinants
contribute to the complex of psychological predispositions producing an
intention to chew or not to chew along with various other factors.
It has been reported in a number of studies that the health-harming behaviors of both smoking and drinking are frequently associated with betel nut chewing (Wang et al. 2003; Tsai et al. 2003). The present study also showed that, although insignificant, both the habits were found with greater frequency in chewers as compared to non-chewers. This suggests that person addicted to one habit, may acquire another one easily due to various inter related socio-psychological and environmental factors.

There was no significant difference between chewers and non-chewers with respect to oral hygiene measures adopted by them. About 87- 90% of subjects in both groups reported use of brush/powder regularly. On the other hand, a mean value of Oral Hygiene Index (OHI) amongst chewers and non-chewers was 2.12 ± 0.86 and 1.54 ± 1.12 respectively. This suggests that the oral hygiene status of chewers was more deteriorated than non-chewers even though both the chewers and non-chewers were undertaking almost same oral hygiene measures. The difference between the two groups was found to be statistically significant (p < 0.001). This discrepancy can be explained on the basis of following reasons. Firstly, the data on oral hygiene measures practiced by the subjects are based on self-reported information by the participants. To the extent that participants may not accurately report the oral hygiene measures employed, they could be misclassified for oral hygiene practices. This would lead to an underestimation of the strength of association between chewing habits and oral hygiene measures practiced in the two groups. Secondly, although the questionnaire revealed whether the individual practiced oral hygiene measures regularly or not, it did not reveal if he/she used brush or powder, how much time was devoted to in brushing or if it was done correctly or not.

Thus, to avoid the misclassification of subjects according to hygiene measures adopted by the two groups, the oral hygiene status of the subjects
was measured using Oral Hygiene Index, which gave the true status of hygiene in the mouth and the data revealed a significantly higher OHI among chewers as compared to non-chewers in the present study. This suggests lower oral hygiene among chewers than the non-chewers.

It has long been suggested that areca chewing may confer some protection against dental caries. The caries rate as analysed with DMF Index was found inversely related to the habit of chewing. At present the factor/s in betel, which result in a lower prevalence of caries, are not well understood. However, in this context, a number of factors could be conceivably important. These include pH of the betel mixture, altered salivary rate and buffering capacity, altered calcium concentrations, the presence of bactericidal or bacteriostatic constituents as well as other agents capable of altering bacterial or tissue metabolism, and, presence of the possibly protective brown-black stain on exposed tooth surfaces. In the present study the mean DMF index was lower among chewers with respect to non-chewers. This suggests that chewing these materials confer some protection against dental caries.

Howden (1984) conducted a study in Papua New Guinea and reported that the amount of dental caries as measured by DMF was considerably and statistically significantly greater for the group of non-chewers (1.162) as compared with chewers (0.364). It was proposed that the betel stain, might mediate its effect by acting as a barrier against acid attack of dental caries. Nigam and Srivastava (1990) also observed that the habit of betel chewing reduces the incidence of dental decay. Miranda et al. (1996) investigated the effects of areca nut and its components on growth of selected microorganisms and found that hydrolysable tannin in tannin fraction, i.e., tannic acid, showed strong antibacterial properties on bacterial growth. They proposed that low prevalence of S. mutans in chewers might, in part, be due to its suppression in the mouth. Keeping in view of these, it is suggested that
antibacterial property of chewing fluid, buffering and alkaline pH might have role in the protection against dental caries. A slightly lower pH of salivary fluid among chewers was also observed in the present study.

In addition to this, chronic chewers also have marked attrition of cusps of teeth leading to loss of occlusal pits and fissures, which may reduce the risk of pit and fissure caries by eliminating potential stagnation areas. The increased production of sclerosed dentine in response to attrition may confer protection against microbial invasion. Furthermore, the process of chewing itself brings copious amounts of saliva to the mouth and in the presence of added slaked lime may increase the pH in the oral environment; this may act as a buffer against acid formed in plaque on teeth. It has also been suggested that the betel stain, which often coats the surface of the teeth, may act as a protective varnish, protecting the tooth against acid attack thereby preventing caries (Howden, 1984).

Thus, most of the data available as well as present study suggests that chewing might offer some protection against dental caries. However, its beneficial effects should be carefully weighed against its serious other adverse health effects including caries. Further, there is no universal agreement among investigators that chewing habit prevents caries. Recently, Tomar and Winn (1999) reported that chewing tobacco might be a risk factor in the development of root surface caries and, to a lesser extent, coronal caries. This may be due to high sugar content, increased gingival recession and enhanced collagenase activity. Another study found no significant difference in caries prevalence between betel nut chewers and non-chewers (Reichart and Gehring, 1984). However, lower dental caries was observed among chewers in the present study. This is in agreement with the earlier findings (Howden, 1984; Nigam and Srivastava, 1990).
The staining of teeth is a striking side effect of betel nut chewing. There is copious production of blood-red saliva that can stain oral structures. After years of betel nut with or without tobacco and catachu chewing, the teeth may become red-brown to nearly black. Extrinsic staining of teeth due to areca deposits is often observed particularly when good oral hygiene prophylaxis is lacking and where regular dental care is minimal. The study showed that the number of chewers having severe staining i.e. score 3 were much higher (16%) as compared to non-chewers which was less than 2%. A similar pattern was found for staining score 2 with 50% subjects in chewers and only 7.1% in the non-chewers group. Moreover, about 63% of non-chewers were not having the stains on teeth as compared to only 3% in chewers. There was significantly more staining in chewers as compared to non-chewers. The severity of staining increased with duration of years of chewing which was also statistically significant. But the frequency of chewing per day did not have any statistically significant role in severity of staining. The ultrastructural features of the black stain have been studied by Reichert et al. (1985) who described the stain as having a composition akin to that of calculus. The more staining of teeth observed among the chewers in the present study is due to chewing habits of these products and also depend upon duration of habits.

There is paucity of data pertaining to the role of areca nut in the causation of attrition. The main direct effects on the hard tissues are due to abrasive action of areca nut on the teeth, which is one of the hardest eatable nuts. The habitual chewing of areca may result in severe wear of incisal and occlusal tooth surfaces, particularly the enamel covering. The loss of enamel may also expose the underlying dentine and as this is softer than enamel, it wears at an increased rate. The exposure of dentine may also result in dentinal sensitivity. The present study clearly indicated a significantly higher number of attrition cases among the chewers than non-chewers. Recently Trivedy et al. (2002) reported that the degree of attrition is dependent upon
several factors, which include the consistency (hardness) of areca, the frequency of chewing and the duration of the habit. Further, excessive abrasion of the incisal and occlusal surfaces of maxillary and mandibular teeth may occur with the habitual use of coarse, abrasive chewing tobacco or cigar (Christen, 1970).

In case of smokeless tobacco, abrasive silica particles found associated with it may contribute to dental attrition in chronic chewers. Although some silica is the result of dust fall out on field grown leaves, much of it comes from soil carried into leaves by plant metabolism. These deposited particles are known as ‘Opaline Phytoliths’ and cannot be removed by any process that would not destroy their tobacco (Bowles et al. (1995). Thus chewing quid containing tobacco might also have a significant role in causation of attrition along with mastication of areca nut on teeth in addition to natural factors.

The present study further indicated that hardness of areca nut might also be responsible for the dental attrition as the number of cases with attrition was also higher among mawa chewers even though its sample size was small. In mawa, the chewers use dried areca nut pieces rather than cured or soft pieces of areca nut which in turn might causes increased wear in the teeth. The direct role of chewing habit in causation of attrition was further indirectly confirmed when attrition was found to increase with increase in duration of chewing, which was statistically significant. Yeh (1997) demonstrated the presence of root fractures in chronic areca chewers and suggested it may be a consequence of the increased masticatory load that is placed upon the teeth and is not a direct effect of areca. This increased masticatory load may also be responsible for higher attrition observed in chewers.
The loss of enamel due to attrition may expose the underlying dentine. Since the dentin contains odontoblastic processes in the dentinal tubules that may convey the various stimuli like cold, hot, etc. to the nerve endings present in the pulp and it results in dentinal sensitivity. In our study, 58.9% chewers complained of sensitivity towards cold beverage compared to 35% in non-chewers, which was statistically significant. Earlier, Irwin and McCuskar (1997) reported prevalence of 57.2% of dentine hypersensitivity in adult patients attending general dental practices. They further reported that cold was the major stimulus for pain followed by brushing, hot and sweet stimuli. Collaert and Fischer (1991) reported that local pulpal inflammation; traumatic oral hygiene and dietary habits have been considered in the etiology of dentine hypersensitivity. They also suggested that dentine hypersensitivity is probably caused by a change in fluid flow in the dentine tubules, which in turn excites the nerve endings located at the pulp-dentine border. The higher sensitivity towards cold beverages observed in the present study could be attributed to excessive load on mastication on tooth due to chewing of tobacco and areca nut. This chewing material might have affected indirectly (excessive load of mastication of chewing material on teeth) the enamel layer covering the dentinal tubules and caused adverse effects on tubules structure and function.

Fragments of areca nuts can become stuck between the teeth, where they may decompose, causing bad breath. This was supported by our observation in chewers as 58.3% complained of bad odor from mouth as compared to 34% in controls. In addition to this, bleeding of gums was significantly more among chewers than non-chewers. Further, the burning sensation of soft tissue was also found to be higher in the chewing group than non-chewing group.

Examination of periodontal health revealed poorer periodontal status in chewers than non-chewers. Pathogenesis of periodontitis in chewers may
involve defective soft tissue metabolism, leading to impaired response. Interactions of various ingredients may act synergistically in the progression of the periodontal disease. However, the precise mechanisms by which areca nut adversely affects periodontal health are still not clear. Chang et al. (1998) reported that areca extracts containing arecoline inhibit growth and attachment of, and protein synthesis in, human cultured periodontal fibroblasts. On the basis of these findings the investigators proposed that areca might be cytotoxic to periodontal fibroblasts and may exacerbate pre-existing periodontal disease as well as impair periodontal reattachment. Earlier, Waerhaug (1967) carried out a survey in Ceylon, Srilanka to assess the prevalence and severity of periodontal conditions in selected populations. He reported that areca consumers had more periodontitis than non-consumers, even when like was compared with like, as far as oral hygiene was considered. He suggested areca nut consumption might act as a factor, which lowers the resistance to local irritants. The present study also indicated deterioration of periodontal condition in chewers. Periodontal pocket, gingival lesions and gingival recession were more among chewers as compared to non-chewers.

A number of investigators have shown that loss of periodontal attachment and calculus formation is greater in areca nut chewers (Mehta et al. 1955; Anerud et al. 1991; Amarasena, 2002). However, it is difficult to interpret such studies, as there are several confounding variables such as the level of oral hygiene, dietary factors, general health and dental status, not to mention tobacco smoking, which may have a significant influence on periodontal status. However, significant deterioration in periodontal condition in chewers than non-chewers suggests a role of chewing habit in periodontal condition.

Leukoplakia is defined as a predominantly white patch or plaque on the oral mucosa that cannot be characterized clinically or pathologically as any
other disease. Based on clinical appearance, leukoplakia can be divided into several subtypes: homogeneous (white), speckled (red/white), nodular or verrucous leukoplakia. It is well known for its potential for malignant change and transformation rates between 3-6% have been quoted in the literature (WHO, 1978). Leukoplakia is considered as one of the precancerous lesions of oral cavity. Earlier, Wahi et al. showed that habit of chewing especially of mainpuri tobacco (a mixture of mainpuri tobacco, lime and areca nut) was associated with a higher prevalence of oral leucoplakia. Further, Mehta et al. (1969; 1972) also reported that chewing betel quid with tobacco was usually associated with higher prevalence of leukoplakia than the chewing of betel quid alone or than no chewing habits.

Although there is considerable debate about how to define oral leukoplakia there is little doubt that both tobacco, in any form, and areca nut use are major risk factors for developing this condition (Gupta et al., 1997; Shiu and Chen, 2004). Analysis of the distribution of leukoplakia cases among chewers and non-chewers in this study revealed that all cases were found in chewers only (5 out of 168 chewers) that were statistically significant. This finding further corroborates the findings of several previous authors that areca nut and chewing tobacco play a significant role in the development and malignant transformation of oral leukoplakia. Earlier, Mehta et al. (1969) conducted cross-sectional surveys of more than 50,000 individuals in five districts of India and found that the prevalence of oral leukoplakia ranged from 0.4% to 1.8% among users of smokeless tobacco as compared with almost zero prevalence of leukoplakia in non users. Gupta et al. (1980), carried out a 10 year follow-up survey of oral lesions in Ernakulam (Kerala), Bhavnagar (Gujarat), and Srikakulam (Andhra Pradesh), in India. The highest incidence was observed in Ernakulam in men who chewed betel quid with tobacco and smoke (6 per 1000), and no new cases were found among those who did not chew or smoked. In the present study, the prevalence of leukoplakia was not observed among the non-chewers.
Recently, Jeng et al. (1999) investigated the effects of areca nut, inflorescence piper betel extracts and arecoline on cytotoxicity, total and unscheduled DNA synthesis in cultured gingival keratinocytes. The results indicated that areca nut, inflorescence piper betel extracts and arecoline take part in the pathogenesis of betel nut chewing-related oral mucosal lesions like leukoplakia, OSMF and oral cancer, possibly through both genotoxic and non-genotoxic mechanisms.

Further evidence of its relationship with areca chewing has come from the increased prevalence of this condition in subjects who suffer from oral submucous fibrosis, which is associated strongly with the habit of areca chewing. The most striking finding of present study was the distribution of OSMF patients among chewers and non-chewers. All of the 36 cases of OSMF were found among chewers. If there are any other equally important factors predisposing to OSMF, it seems likely that their causative influence would have produced the lesion in at least some subjects not habituated to areca nut and tobacco chewing. Although the lesion had almost equal distribution among various chewing groups using quid with tobacco, without tobacco or those with mixed habits. However, there were greater numbers of cases observed with severe OSMF in subjects who included tobacco in the quid. This may be due to the fact that tobacco and areca nut may act synergistically to increase the severity of the lesion. However, there was no particular correlation between prevalence of OSMF and any particular chewing product e.g. mawa, khaini, gutkha etc. Further, no significant difference in the severity of OSMF was observed in relation to chewing frequency per day or the duration of chewing habit. Thus, it must be kept in mind that the disease progress is not dictated solely by the duration or frequency of exposure to it or by simple process of passive diffusion. There may be additional co-factors necessary for the development of OSMF. Many investigators have described areca nut chewing as the most important factor.
for OSMF (Bhonsle et al. 1987; Lu et al. 1993). Several case-control studies have also shown that there is an increased risk of developing OSMF in subjects consuming areca products (Shiau and Kwan, 1979; Pindborg et al. 1980). The evidence from the literature as well as data of the present study, strongly implicates areca in the etiopathogenesis of OSMF although further studies are warranted to uncover the mechanisms underlying the pathological processes in relation to the development of the fibrosis and transformation to squamous cell carcinoma, which can be considered as the most serious oral health consequence. Very recently, Ranganathan (2004) reported that all areca nut products were associated with OSMF, with the risk being greater for pan masala. They also reported that the duration of the habit was more significantly associated than the frequency of the chewing habit.

OSMF patients present with the major complaint of progressive difficulty in achieving adequate mouth opening which might be due to the accumulation of inelastic fibrous tissue in juxta-epithelial region of oral mucosa. The significant lower distance between upper and lower interincisor and intermolar among OSMF cases and non-chewers was observed in the present study. The fibrosis may lead to difficulty in mastication, speech and swallowing (if esophagus gets involved). The burning sensation especially on eating highly seasoned foods is also observed. In the advanced cases the jaws may not separate. The inelastic fibrotic mucosa is forced against the edges of teeth causing ulceration, which may become secondarily infected (Canniff et al. 1986). In the study, OSMF was found in 21.4% chewers and none among the non-chewers. It is now accepted that chewing areca is the single most important etiological factor for developing OSMF. Other causes, which have been proposed in the past but have not been substantiated include excessive consumption of chillies, autoimmune reaction and nutritional factors, particularly iron deficiency etc (Murti et al. 1995).
Meghji et al. (1982) in their study concluded that tannins from chewed areca nuts might enhance the development of fibrosis in OSMF by reducing the susceptibility of collagen to degradation by collagenase. In vitro studies have shown that extracts of areca nut may stimulate cultured fibroblasts to proliferate and synthesize collagen and this might be involved in the etiology of OSMF (Canniff and Harvey, 1981; Harvey et al. 1986). However, recent studies have shown that arecoline inhibits collagen synthesis and fibroblast proliferation in vitro, suggesting that arecoline may have cytotoxic properties (Chang et al. 1998). The disparity of results from in vitro studies suggests that the areca may contain other agents that are important in the pathogenesis of OSMF. Further, studies are needed on this aspect to reach the conclusion.

Lysyl oxidase, an extracellular enzyme, is responsible for cross-linkage of collagen that may become more resistant to digestion by collagenase. When compared with fibroblasts from normal mucosa, elevating of lysyl oxidase in OSMF fibroblasts is noted that contribute to collagen deposition (Ma et al. 1995). Presence of high levels of copper may also up-regulate the lysyl oxidase activity, leading to excessive cross-linkage and deposition of collagen (Trivedy et al. 1997). Later they also reported higher copper level in submucous fibrotic tissue as compared to normal tissues (Trivedy et al. 2000).

The disease is clearly multifactorial in origin as only few chewers developed OSMF. Thus there are some other co-factors that are necessary for the development of OSMF such as lack of antifibrotic activity and genetic susceptibility etc. It seems that the disease has a genetic predisposition (HLA-linked), which renders the oral mucosa susceptible to inflammatory changes if they chew betel nut (Canniff et al. 1985). This would explain why it was observed in few patients with little exposure to areca nut and not in heavy chewers. Further, Canniff et al. (1985) demonstrated a genetic predisposition to the disease, involving raised frequencies of HLA antigens A10, DR3.
Complaints of difficulty in mouth opening (which were further corroborated by significantly reduced inter-incisor and inter-molar distances in chewers), difficulty in swallowing and burning sensation in soft tissue of mouth – all symptoms suspected to be associated with OSMF and damage to oral soft tissues due to chewing habit were significantly associated with chewers compared to non-chewers.

Association of lichen planus with chewing habits is not yet clear or even controversial. Direct correlation has not been proved although a number of studies have shown prevalence of lichen planus and lichen planus like lesions in the oral cavity of chewers. Ikeda et al. (1995) studied the epidemiology of oral mucosal lesions in a selected Cambodian population. They found that all subjects with lichen lesions were women. The age-adjusted relative risk for developing lichen planus among betel nut chewers as compared to non-chewers was 3.3. Gupta et al. (1997) compared the incidence rates amongst those who reportedly stopped their betel-quid chewing habit versus those who did not, and demonstrated that leukoplakia, oral submucous fibrosis and oral lichen planus-like lesion were directly associated with the betel quid chewing habit while oral lichen planus was not. In our study, lichen planus was observed in non-chewers also. Two subjects out of 197 non-chewers i.e. (1.0%) and 2 subjects out of 168 chewers (1.19%) had lichen planus. Thus the data suggest that there are other factors, which may be responsible for lichen planus.

Diagnostic cytopathology is a useful and well-established clinical tool in medicine. Because the epithelial surfaces continuously shed cellular material, microscopic analysis of released cells frequently provides useful information regarding the status of the tissue from which they originate. For a long time, it is known that inflammatory, infectious, premalignant and malignant cellular features are often identifiable through such microscopic analysis. However, it
has recently been recognized that similar studies can be used to monitor and detect early biologic changes in specific tissues due to exposure to potential mutagenic and carcinogenic chemicals. The micronucleus (MN) test is one such indirect and sensitive measure of chromosomal breakage or missegregation that has received increased attention as a sensitive biologic marker of genotoxic exposure. In this study, MN test was used to determine if the use of areca nut and smokeless tobacco caused any genetic injury in buccal mucosal cells of the chewers. Microscopic analysis of the Feulgen-stained cells from chewers and non-chewers showed clear quantitative difference with respect to micronucleus. The cells sampled from buccal mucosa of chewers showed a higher frequency of cells with micronuclei than the non-chewers. Stich et al. (1982a,b, 1983) also reported elevated frequency of micronuclei in buccal mucosa cells of users of Khaini tobacco and areca nut as compared to non-chewers and concluded that it is an indirect evidence of cytogenic damage in the tissue at high risk of developing cancer. Later, Adhvaryu et al. (1991) also found statistically significant rise in the frequency of micronuclei in exfoliated oral mucosa cells of normal chewers, chewers with OSMF and chewers with cancer as compared to controls. Further, Kayal et al. (1993) analyzed the frequencies of micronucleated cells in the buccal mucosa of normal healthy individual who were regularly using various chewing products and also among the OSMF patients. They have reported significantly higher frequencies of MN in all type of chewers than non-chewers. In the present study using student ‘t’ test showed that chewers having habit for > 20 years had significant higher risk for micronuclei induction in the oral buccal mucosa in comparison to non-chewers. Further non-parametric test (Kruskall-Wallis one way analysis of variance) indicated that not only chewers of areca nut and tobacco products were having significantly higher risk of inducing MN in buccal mucosal cells but duration of chewing also had a significant impact. The data available as well as results of present study clearly indicates that chewing of areca nut and
tobacco has a significant role in the induction of micronuclei in the buccal mucosa cells of chewers.

Role of pH on the mutagenic activity of areca nut preparations was demonstrated by Rosin (1984). He observed that an aqueous extract and a tannin containing fraction of areca nut induced gene conversion in *Saccharomyces cerevisiae* D7 at alkaline but not at acidic pH in the absence of an exogenous metabolic system. In a study carried out Assamese betel nut (Tamol) with betel leaf, lime and tobacco brought changes in the salivary pH level considerably (towards alkalinity) during chewing. A slow change towards neutral pH was seen after spitting out of macerated quid (Kumar et al. unpublished). The present data also indicated that pH level of chewers was slightly alkaline than that of non-chewers. Thus salivary alkaline pH might have role in causing genotoxicity of areca nut products.

The oral mucosa located at the anatomic sites sampled in the study is usually nonkeratinized. The epithelium is composed of four strata: basal layer, prickle cell layer, intermediate and superficial layers. The oral epithelium maintains itself by a system of continuous cell renewal in which new cells produced by mitosis in the basal layer migrate to the surface to replace those that are shed. The turnover time required for a cell to divide and migrate through the epithelium to the superficial layer is about 25 days. Furthermore, the buccal and labial mucosa is only about 500 μm thick (Ten Cate, 1985). Thus, it is possible that saliva-soluble compounds from the quid could diffuse to the basal layer and disturb the reproductive mechanism of the proliferating cell population. The direct contact of the quid with the mucosa is a chronic source of physical and chemical irritation, which could be partly responsible for cellular response observed. Although the mechanism of action is still not well understood, areca nut and tobacco clearly appear to induce nuclear alterations in the epithelial cells of oral mucosa. These nuclear alterations
may reflect a change in pattern of cellular maturation or perhaps a more direct karyolitic action of the components on the nucleus of the epithelial cells. It is also possible that the changes reflect the observed keratinization of the superficial layer of buccal mucosa. Recently Ramesh et al. (1999) reported that the use of tobacco influences the cytomorphology of the normal buccal mucosa. Betel chewing with tobacco influences the nuclear and cell diameters, while smoking influences only the nuclear diameter. Further they also suggested that cell and nuclear diameters could possibly be sensitive parameters in the diagnosis of oral premalignant and malignant lesions (Ramesh et al. 1998). The data available as well as present study suggest that areca nut and tobacco damages the genetic integrity of the cells in the oral mucosa, this mechanism of action may explain the way these products are involved in the pathogenesis of oral cancer. Recently, Nigam et al. (2001) conducted an experimental study to find out the role of pan masala plain and pan masala gutkha in the causation of cancer. They reported the carcinogenic potential of pan masala plain as well as with tobacco (gutkha) in the mouse.

In recent years, copper has been implicated in pathogenesis of OSMF. High level of copper is found in the areca nut as compared to other nuts (Trivedy et al. 1997). The results of the present study also indicated an appreciable amount of copper in the different areca nut preparations such as pan masala and areca nut, which are used in this part of the world. Further, marginally higher level of copper was also noted in the serum of chewers as compared to non-chewers. However, these changes were statistically not significant. Jayadeep et al. (1997) found that copper was significantly increased in oral leukoplakia and cancer. They further reported that the level of zinc decreased significantly only in male patients with leukoplakia and cancer. Trivedy et al. (1999) proposed that the high copper content of the areca nut might play an important role in the etiopathogenesis of OSMF. Later they reported raised copper levels in oral biopsies from patients with OSMF (Trivedy et al. 2000). In the present study a slightly higher level of copper was
observed in the saliva of the OSMF subjects than chewers and non-chewers. However, slightly lower level of copper was noticed in the urinary sample of OSMF subjects than chewers and non-chewers. This might be due to the accumulation of copper in tissues of OSMF subjects; thereby lower level of copper is available for excretion through urine among the OSMF subjects. Recently Trivedy et al. (2001) also reported that the addition of copper to fibroblasts at concentrations compatible with that found in saliva after chewing areca nut could cause a significant increase in the synthesis of collagen. This suggests a possible role of copper in the OSMF.

The role of copper cannot be segregated from that of zinc, the biochemical relatedness with which is well elucidated. The transport of copper into the oral epithelium may be dictated more by the composition of the quid rather than the time of exposure. Zinc is implicated in the modulation of mucosal metallothionein, thereby interfering with copper absorption. The bioavailability of zinc in its turn depends on elements like calcium and iron present in oral fluid. The usage of slaked lime (calcium hydroxide) as an ingredient of betel quid, thereby causing an interference with zinc bioavailability is a matter of concern (Rajendran and Karunakaran, 2002). The presence of high concentration of copper (as is seen in areca) may also diminish zinc transport (ATSDR, 1994). Although direct data pertaining to this type of interaction is scanty, there is at least one study by Scott and Turnlund (1994) that compared zinc kinetics in subjects with normal, high or low copper intake. They also investigated sites of interaction between copper and zinc and the degree of interaction at each site. They reported that zinc absorption was lower on high copper intake (28% versus 34%, respectively).

Varghese et al. (1987) reported a significant reduction in the serum zinc levels in both oral submucous fibrosis and oral cancer. The copper/zinc ratio was also found to be elevated in oral submucous fibrosis and depressed in oral cancer. Kumar et al. (1991) conducted a clinical study to evaluate the possible therapeutic role of zinc in the treatment of OSMF and found
significant decrease in the severity of the disease following oral supplementation of zinc. The study signifies the role of deficiency of zinc in OSMF patients. Our study also found higher zinc level in non-chewers followed by chewers and OSMF patients. A similar trend was also observed in saliva zinc level.

Interactions of chemicals, metals, drugs, etc. with the immune system may affect the human health. The prime concern is to assess the importance of these interactions with regard to its impact on general state of well being. The toxic response may occur when the immune system is the target of chemicals resulting in altered immune function; this in turn can result in decreased resistance to infection, certain forms of neoplasia, or immune dysregulation or stimulation which exacerbates allergy or autoimmunity. Alternately, toxicity may arise when the immune system responds to the antigenic specificity of the chemical as part of a specific immune response (allergy or autoimmunity). Certain agents induce autoimmunity. The detection of immune changes on exposure to various agents is more complicated and difficult in humans than in experimental animals. The studies reported have several constrains considering confounding factors such as age, sex, race, gender, co-existence of disease, food etc (Karnik, 2001). Although a number of studies have been conducted to study the relationship between tobacco consumption and humoral immunity, most of these are concerned with smoking and the results have been controversial.

Data regarding influence of smokeless tobacco or areca nut on serum immunoglobulin is rare. Moszczynski et al. (2001) studied the effect of tobacco smoke on humoral immunity and reported a decrease in serum IgA, IgG and IgM in smokers as compared to non-smokers. Similar findings have been reported by Mili et al. (1991). Andersen et al. (1982) found lowered levels of IgG and IgA in smokers. The mean serum IgM values were not
significantly different between smokers and non-smokers. On the other hand, Zetterstrom et al. (1985) demonstrated that tobacco smoke did not significantly affect the serum IgM and IgG. Shah et al. (1994) conducted a study to evaluate the possible role of immunological factors in the pathogenesis of OSMF and reported significantly elevated levels of salivary IgA, IgG and IgM in patients with OSMF. In this study, mean IgG level was found to be raised in chewers as compared to non-chewers whereas IgA was lowered in chewers. The IgM levels were almost similar in both the groups. This difference indicates that constituents of tobacco entering the bloodstream of smokers differ from those of areca nut and smokeless tobacco users. Since nicotine and its metabolite, cotinine, are found in blood of smokers and chewers at comparable levels (US Dept. Health and Human Services, 1986), these alkaloids do not appear to be offending agents in this case. The difference may, however, be due to pyrogenic substances produced during tobacco combustion, which affect only smokers. Thus, the mechanism of influence of chewing habit on humoral immunity appears to be intricate and further studies are required to ascertain its role.

It is evident from foregoing discussion as well as from the present study that the chewing habits may be clearly implicated in etiopathogenesis of various premalignant lesions such as oral leukoplakia, submucous fibrosis etc. The genotoxic potential of areca nut and tobacco is now well established. An international working group of scientific experts convened by the Monographs Programme of the International Agency for Research on Cancer (IARC), part of the World Health Organization, has reviewed the published studies related to cancer and chewing betel quid and areca nut. A previous evaluation in 1985 had found that chewing betel quid with tobacco is carcinogenic to humans. The new evaluation goes further to conclude that chewing betel quid without tobacco is also carcinogenic to humans. The working group also suggested that the areca nut, a common component of many different chewing habits, is carcinogenic to humans. Trivedy et al.
(2002) suggested that areca nut might have a potentially harmful effect on the oral cavity. It is a risk factor for oral cancer, oral cancer recurrence, adult periodontal diseases, suppresses the immune system's response to oral infection, retards healing following oral surgical and accidental wounding, promotes periodontal degeneration in diabetics and adversely affects the cardiovascular system.

The data from the study indicated adverse effects on hard tissues in the oral cavity in the form of higher attrition and sensitivity among chewers. Further, it was found that the chewing habits adversely affected the oral soft tissues. The periodontal condition was deteriorated and precancerous lesions like leukoplakia and oral submucous fibrosis were found only among the chewers. The direct genotoxic damage to oral mucosal cells was also evident in the form of increased frequency of micronuclei among chewers as compared to non-chewers. Thus, the present study clearly indicates that areca nut and pan masala with or without tobacco have deleterious effects on oral hard and soft tissues.