Review of Literature
The custom of chewing betel nut or areca nut/betel leaf is an ancient one extending back in time to at least several centuries BC. The habit probably originated in the region of the Indian subcontinent and has spread to South-East Asian countries and later extended to some other parts of the globe. Chroniclers recorded the widespread popularity of the custom especially in Indian subcontinent. The habit retains its popularity or may even have increased in recent years, as various areca nut products are available commercially now, which can be stored and carried easily. Betel nut chewing related oral mucosal lesions are potential hazard to a large population worldwide. It is estimated that about 600 million people worldwide currently subscribe to the habit of chewing areca nut (Warnakulasuriya and Peters, 2002). Countries where considerable areca nut chewing is encountered are Kampuchea, Vietnam, Thailand, Taiwan, Laos, Southern parts of China, India, Pakistan, Sri Lanka, Bangladesh, Burma, Nepal, Malaysia, Singapore, Philippines, Indonesia, Papua New Guinea, and associated island groups. Betel quid usage may also be encountered in Indian communities residing in some African countries such as Uganda, South Africa, Tanzania and Kenya. Similarly, betel quid chewing habits are also found among Indian communities in Pacific countries such as Fiji etc.

Because of the variable nature of the constituents of the quid as well as difference in the method of preparation of quid, some terminological discrepancies are encountered in descriptions of this practice around the world. Various terms such as ‘Betel nut’, ‘Betel quid’, ‘Betel’, ‘Betel nut habit’. 
Tobacco chewing, 'Pan chewing', 'Pan masala', ‘Gutkha’, ‘Mawa’, ‘Tamol,’ ‘Kwai’ etc have been used by various investigators to describe the habit. However, areca nut is the major constituent of all these preparations. In most of the chewing quid, areca nut constitutes more than 60-80 % of total weight of the quid. The habits of betel nut chewing are different in various parts of the world including different parts of India. In Assam, one of the Northeastern states of India, raw betel nut with fibrous pericarp of the fruit is kept for a period of about 3 to 4 months in an underground pit. After that the fibrous pericarp is separated from the seed or endosperm, which is then used (locally known as Tamul) along with betel leaf with or without lime and tobacco. The use of other ingredients is rare in this part of country. Some of the local people have as much as 30 to 40 quid per day. The chew quid is swallowed, spat out or kept on one side of the mouth and some habitual chewers may even sleep with quid in their mouth. In Western India, especially in Gujarat state, people use dried betel nut with lime and tobacco, which is commonly known as Mawa. In South India, the habit of using raw betel nut with betel leaf with or without tobacco and lime is prevalent. However in North India, and most of the other parts of the India, use of pan is encountered, which contains betel leaf, lime, catechu, areca nut with or without tobacco and certain other ingredients. In Meghalaya, India, people chew ‘Kwai’, which contains raw areca nut, lime with or without tobacco and some people use a piece of ginger also with it. Mixture of pieces of areca nut with small amount of tobacco flakes and a few drops slaked lime is used in Maharashtra, locally known as ‘Karra’. Pindborg et al. (1967), surveyed several countries and found no less than 30 different ways of chewing tobacco and/or betel nut.

A new habit of chewing pan masala with tobacco (locally known as gutkha) or without tobacco (pan masala sada or plain) is gaining popularity in Indian subcontinent as an alternative to habit of tobacco chewing. It is particularly evident in youth population and may be practiced even by those who generally refrain from smoking and tobacco chewing. Pan masala
consists of areca nut (*Areca catechu*), catechu (*Acacia catechu*), lime, cardamom (*Elettaria cardamomum*), and unspecified flavoring agents.

Chemical analysis of different types of pan masala (plain as well as with tobacco) showed the presence of polycyclic aromatic hydrocarbons, nitrosamines, toxic metals and residual pesticides (NIOH, 1989). Some of them are potentially cytotoxic and genotoxic and may play a role in causation of oral as well as certain other systemic diseases. More than 200 brands of pan masala products are available in India. Pan masala gutkha has been targeted towards youth and has become extremely popular. An evolving epidemic of oral submucous fibrosis attributed to gutkha use has been documented among youth, with a resultant increase in oral cancer in lower age groups (Gupta and Ray, 2002).

Haemodynamic effects of pan masala in healthy volunteers have been studied by Sharma et al. (2000). They concluded that pan masala without tobacco intake causes acute increase in pulse and blood pressure after 10 minutes of consumption. The prime suspect for betel nut carcinogenesis is alkaloids, a group of reduced pyridine compounds producing various adducts including cysteine $\beta$-alkylation products (Sharan, 1996). Arecoline (1,2,4,5-tetrahydro-1-methyl-pyridine carboxylic acid) is one of the most abundant alkaloid of betel nut, but other alkaloids such as arecaidine (1,2,5,6 tetrahydro-1-methyl-3-pyridine carboxylic acidid), guavacoline (methyl ester of guavacine), arecolidine are also present in small or trace amounts (Sharan, 1996). He also mentioned that arecaidine is reported to be more toxic than arecoline but both have similar pharmacokinetic behaviour. Further, the chewing of betel quid with tobacco leads not only to the formation of TSNA (tobacco specific N- nitrosamines) but also to the formation of arecoline derived nitrosamines, namely, N-nitrosoguavacine and N-nitrosoguavacoline, and the highly carcinogenic 3-(methylnitrosoamino) propionitrile (Nair et al. 1986; Prokopezyk et al. 1987). Very recently Kumpawat et al. (2003)
observed that aqueous extract of raw betel nut has genotoxic properties, which is further enhanced by depletion of endogenous glutathione level. Further, this study indicates that the generation of ROS by arecoline could partially contribute to the induction of chromosomal aberrations (CAs), since the frequency of arecoline induced CAs was reduced either by post treatment with super-oxide dismutase or in anoxic conditions. The areca alkaloids act as competitive inhibitors of GABA (gamma- amino butyric acid) receptors and have widespread effects in the body, including actions on the brain, cardiovascular system, lungs, gut and pancreas. Nitrosated derivatives of areca alkaloids induce tumors throughout the upper gut and fore gut in animals and are also associated with increased risk of tumors in man. (Boucher and Mannan, 2002). The effects of chronic betel nut usage in man are at least as diverse as those of smoking. The diseases caused by areca nut with or without tobacco chewing place an extra burden on developing countries especially of South-east Asian region already beset by malnutrition and communicable diseases.

Tobacco has been chewed for centuries and possibly used for millennia. At first only the native populations of America chewed it, subsequently, tobacco was brought to Europe in the middle of the sixteenth century, smoking/chewing became widespread throughout the world. Following the introduction of tobacco into India by the Portuguese in late sixteenth century, its use spread rapidly to all parts of the country, percolating into all sections of the society. Betel quid and areca nut chewing are widely practiced in various communities in the world, with hundreds of millions of users worldwide. Betel quid is chewed for many reasons, such as its stimulant effects, to satisfy hunger, to sweeten the breath, and as a social and cultural practice (WHO, 2003). What is incontrovertible is that once smokers/chewers have become accustomed to its effects, prolonged abstention is distressing, and the feeling of distress can be relieved by further use (IARC, 1986). It has
been estimated that among men, 19-40% of all deaths and among women, at least 4% of all deaths are caused by tobacco in India (Ray et al. 2003).

Tobacco is perhaps the most important carcinogen known to mankind. Tobacco smoking and chewing habits have been casually linked to chronic disability and death from a wide range of neoplastic, cardiovascular and respiratory disease (Doll, 1986). Peterson (2003) reported that in addition to several other chronic diseases, tobacco use is a primary cause of many oral diseases and adverse oral conditions such as oral cancer, periodontal disease and congenital defects in children whose mothers smoke during pregnancy. He also mentioned that the epidemic of tobacco use is one of the greatest threats to the global health and approximately one-third of the adult population in the world use tobacco in some forms and of whom half will die prematurely. According to the most recent estimate by the WHO, 4.9 million people worldwide died in 2000 as a result of their addiction to nicotine (WHO, World Health Report, 2002). The pattern of tobacco usage in western countries like United States, Sweden and third world countries like India, Pakistan has changed dramatically in the past two decades. While the numbers of cigarette smokers have declined, smokeless tobacco use (chewing tobacco) has increased, particularly among male adolescents (Hsu et al. 1980). Rani et al. (2003) conducted a cross sectional, nationally representative population based household survey in India and reported that about 16% of the study population smoked tobacco; 20% chewed tobacco/pan masala; 30% (46.5 % men and 13.8% women) was involved in either smoking or chewing tobacco. These figures translate to 194 million people aged 15 years and above (150 million men and 44 million women) in India who may be using tobacco in some form. The rapid increase in the use of smokeless tobacco is due to the popularity of commercially available products, as well as availability in polythene pouches suitable to store and carry in pockets and also social acceptance of the products (IARC, 1985, 1986). Also, it is a popular myth that the products without tobacco such as
plain pan masala are a safe alternative to betel quid with tobacco. The evidence shows that use of these products cannot be considered safe since these products contain various ingredients reported to have toxic effects. Several states in India have begun to regulate these products, and reductions in oral disease and oral cancer can be expected to follow from reduction in their use (WHO, 2003).

In comparison to smoking habits, the patterns of use of smokeless tobacco are less documented, particularly in developing countries (Gupta and Warnakulasurya, 2002; Mackay and Erikson, 2002). It is firmly established that tobacco use is a prime cause of many oral diseases and adverse oral conditions (Johnson and Bain, 2000; Reibel, 2003). Further Chang et al. (2001) suggested that the compounds of tobacco products might act synergistically in the pathogenesis of oral mucosal lesions in the areca quid chewers. In addition to local effects, areca is also known to exert certain systemic effects. Betel nut chewing has been claimed to produce a sense of well-being, euphoria, heightened alertness, sweating, salivation, a hot sensation in the body and increased capacity to work. Betel nut chewing also leads to habituation, addiction and withdrawal. However, the mechanisms underlying these effects remain poorly understood. Arecoline, the major alkaloid of areca nut, has been extensively studied, and several effects of betel nut chewing are thought to be related to this component of quid. Both sympathetic and parasympathetic nervous systems appear affected, with modulation of both cholinergic and monoamine transmission. However, betel chewing may produce complex reactions and interactions. In the presence of lime, arecoline and guavacoline of areca nut are hydrolyzed into arecaidine and guavaccine, respectively, which are strong inhibitors of GABA uptake. Piper betel flower or leaf contains aromatic phenolic compounds, which have been found to stimulate the release of catecholamines in vitro. Thus, betel chewing may affect parasympathetic, GABAnergic and sympathetic functions. Betel chewing produces an increase in heart rate, blood pressure, sweating
and body temperature. It also increases plasma concentrations of norepinephrine and epinephrine. These results suggest that betel chewing affects both central and autonomic nervous systems (Chu, 2001).

Knowledge on the relationship between betel nut usage and variety of oral pathosis are based primarily on epidemiological studies. It can be theoretically hypothesized that chewing of areca nut might produce adverse effects on both soft and hard tissues of oral cavity. Further, it is also possible that these chewing habits may also affect other organs system as some of the chewers swallowed the chewed material and absorption of chemical constituents from the quid may occur through the oral mucosa. However, it is difficult to pinpoint adverse effects based on individual components of quid as most of the chewers used quid containing several ingredients. An attempt has been made to review all the available information on the subject relevant to this thesis. The present review is divided in to two different parts on the basis of effects of quid on hard and soft tissues and also includes data available on genotoxic potential as well as role of certain metals especially copper in oral diseases like oral submucous fibrosis.

**Chewing habits and hard tissues:**

Areca nut, which is one of the hardest nuts, might affect dentine structure and function due to excessive load of mastication on the teeth. However, data pertaining to role of areca nut on hard tissues in the form of dental caries, attrition and sensitivity are not available or very scanty. Some contradictory reports are available on role of areca nut chewing on dental caries. Some of the studies suggested that areca nut chewing might confer protection against dental caries. Epidemiological studies carried out in South East Asia suggest that the prevalence of dental caries in areca chewers is lower than non-chewers. (Shourie, 1948; Chandra and Desai, 1970; Nigam and Srivastava, 1990). However, some studies have shown that there is no difference in the prevalence of dental caries between areca chewers and non-
chewers (Reichart and Gehring, 1984; Williams et al. 1996). There are reports that constituents of smokeless tobacco might predispose or act as etiological factors in the development of dental caries (Tomar and Winn, 1999). It is also known that various forms of smokeless tobacco contain fluoride, which could well offer some protection against caries (Going et al. 1980), both directly to the teeth and also via the dental plaque. While some products contain caries-promoting fermentable carbohydrates (Hsu et al. 1980), others use non-fermentable artificial sweeteners, which would not increase the plaque’s potential to produce caries-inducing acids. The differences in the constituents of commercially available smokeless tobacco products might be responsible for the inconsistent results obtained by various investigators (Going et al. 1980; Hsu et al. 1980; WHO, 1988).

Habitual chewing of areca nut may result in severe wear of incisal and occlusal tooth surfaces, particularly the enamel. This loss of enamel may expose the underlying dentin to the chewing material, which being softer wears at an accelerated rate. Since the dentin also contains dentinal tubules its exposure will lead to increased sensitivity perceived by the subject to hot and cold. Thus it is hypothesized that hardness of areca nut and coarse tobacco in the chewing products might have role in the causation of attrition as well as sensitivity towards hot and cold food stuffs and beverages. The degree of attrition might depend upon various factors like consistency of areca nut, frequency and duration of the habit as well as other dietary and natural factors such as ageing and structure of teeth etc. Very recently Yin et al. (2003) conducted a clinical study on the relationship between tooth abrasion and the habits of betel nut chewing. They reported that occlusal surfaces of all patients had abrasion from mild to severe. The longer the chewing time, the more severe the occlusal abrasion. The occlusal abrasion became more severe with the increase in chewing frequency. Root fractures have also been demonstrated in chronic areca chewers and this is likely to be a consequence of increased masticatory stresses placed on teeth and as
such is not a direct effect of areca nut suggested by Yeh (1997). It has been reported that excessive tooth surface wear may occur at the site of tobacco use (WHO, 1988). However, such evidence as exists is related mainly to tobacco chewers rather than areca nut chewers, and surface wear is probably the result of the mechanical abrasion caused by the chewing action. However, practically mechanical abrasion is more while chewing of areca nut compared to tobacco chewing.

**Effects on Periodontal Tissues:**

Less attention has been paid regarding association of smokeless tobacco, chewing habits and the health of gingival and periodontal tissues. Further, it is also difficult to correlate the lesions of periodontal tissues with the individual component of quid because of variation in study design and diagnostic criteria used by various workers. Thus the effects of chewing habit in relation to periodontium are not yet clearly established (WHO, 1988). However, Jeng *et al.* (1999) conducted an *in vitro* study reported that arecoline and areca nut extract suppressed the growth of cultured gingival keratinocytes. Earlier, Chang *et al.* (1998) had demonstrated that areca nut extracts that contain arecoline inhibit growth, attachment and protein synthesis in cultured human periodontal fibroblasts respectively. They suggested that areca nut might be cytotoxic to periodontal fibroblasts and thus exacerbate preexisting periodontal diseases as well as impair periodontal reattachment. Loss of periodontal attachment and calculus formation has been found to be greater in areca nut chewers as reported by Anerud *et al.* (1991). But it is difficult to interpret such studies, as there are several confounding variables such as dietary factors, oral hygiene status, smoking etc. that might have a significant influence on periodontal status. The first study demonstrating a detrimental influence of betel chewing on the periodontal tissues was carried out by Mehta *et al.* (1955). They found a higher prevalence of periodontal disease among betel chewers than non-chewers. Gupta (1964), who examined 1673 persons in Trivendrum, Kerala,
in South India found that the mean periodontal index (PI) for those who chewed betel nut was consistently greater than for those who did not chew, suggesting role of chewing in periodontal health. Waerhaug (1967) carried out a comprehensive survey among 8217 persons aged 13-60 years in Sri Lanka; about thirty percent of them had the habit of betel chewing. It was found that betel nut chewers over the age of 20 years had a very high PI indicating greater periodontal breakdown among chewers than non-chewers, even when subgroups of equivalent oral hygiene were compared.

However, gingival recession at the site of placement is common among teenagers of smokeless tobacco users (WHO, 1988). In addition, 77-87% of those who have gingival recession also have evidence of related oral mucosal pathology (WHO, 1988). Such soft-tissue changes are also found at the site of placement (Greer and Polson, 1983). It is therefore difficult to ascertain the effects of areca nut and tobacco chewing on periodontal health but recent in vitro studies of Chang et al. (1998) and Jeng et al. (1999) point towards the adverse effects of areca nut on the gingiva and periodontium.

Dentin hypersensitivity is a common painful condition of the teeth for which little is known of the etiology and predisposing factors. Erosive agents are probably responsible for initiating sensitivity by opening dentinal tubules. Addy and West (1994) mentioned that abrasive and erosive factors, by their effects on enamel and gingival, are important in localizing sites of exposed dentine. They further emphasized that there is a need for further research into understanding the condition itself and its etiology. Collaret and Fischer (1991) suggested that dentin hypersensitivity is probably caused by a change in fluid flow in the dentinal tubules, which in turn excites the nerve endings located at the pulp-dentin border. Al-Wahadni and Linden (2002) reported that dentin hypersensitivity is associated with gingival recession. Further, they also mentioned that infrequent tooth brushing and smoking were also factors.
associated with dentin hypersensitivity. Very little is known about relation between smokeless tobacco and areca nut chewing and dentin sensitivity.

**Oral leukoplakia:**

Oral leukoplakia has been widely regarded as a pre-cancerous lesion and term literally means a white patch (Gr. *Leucos* = white; *plakia* = patch). Leukoplakia is defined as 'A raised white patch of the oral mucosa measuring 5 mm or more which can not be scrapped off and which cannot be attributed clinically or pathologically to any other diagnosable disease' (WHO, 1978). Oral leukoplakia and other oral lesions were reported at the habitual sites of placement of betel quid and smokeless tobacco, including the buccal mucosa and groove, labial mucosa and groove, gingivae, anterior two-thirds of the tongue and floor of the mouth (Mehta *et al.* 1969). Various studies have been conducted to establish the relationship between these products and oral leukoplakia (Mehta *et al.* 1971, 1972; Pindborg *et al.* 1967; Wahi *et al.* 1970; Gupta, 1984). However, data on oral leukoplakia with respect to different components of chewing material are scanty except the close association between tobacco chewing and oral leukoplakia. Higher prevalence of leukoplakia was found in two studies in subjects who included tobacco in the betel quid (Mehta *et al.* 1969; Wahi *et al.* 1970). However, owing to the varieties of habits prevalent in different parts of the world, it is often difficult to find sufficiently large number of subjects with the single habit. Mehta *et al.* (1969, 1972) carried out house-to-house survey in various villages in India. Leukoplakia was usually associated with higher prevalence of chewing of betel quid with tobacco than chewing of betel quid alone or than no chewing habit. Earlier Wahi *et al.* (1970) also showed that habit of chewing was associated with higher prevalence of leukoplakia than no chewing habit. No distinction was made between those chewing betel nut alone and those chewing betel nut with tobacco. 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 smoked (6 per 1000), and no new cases were found among those who did not chew or smoked. They further reported that the annual rates of malignant transformation of leukoplakia were 3.9 per 1000 men per year and 6.01 per 1000 women per year. Gupta (1984) reported a positive dose-response relationship between tobacco habit and the prevalence of leukoplakia. 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. The results of available studies support the hypothesis that smokeless tobacco use plays a significant role in the development and malignant transformation of oral leukoplakia, as also documented in earlier studies (Mehta et al. 1971; IARC, 1985; NIH, 1986). Earlier, the relationship between prevalence of oral leukoplakia and type of smokeless tobacco chewed was studied by Wahi et al. (1970). It was found that prevalence of leukoplakia correlated well with tobacco chewing but varied with the type of tobacco chewing. Daily chewers were 60 times more at risk of developing oral leukoplakia as compared to non-chewers. Several other risk factors found in the study were increased frequency of chewing, early initiation of the habit and duration of the exposure to quid.

The most convincing evidence for the etiological role of tobacco came from intervention studies which demonstrated that leukoplakia regressed significantly more often when tobacco habits were discontinued or reduced compared to when the habits remained unchanged (Mehta et al. 1982). This evidence is supported by other studies also (Mancini et al. 1965; Hirayama, 1966, Martin et al. 1999). Further, a significant fall in the incidence rates of leukoplakia in the intervention cohort was also reported where special health education regarding the ill effects of tobacco usage was provided, compared to the incidence in the control (Gupta et al. 1986). Kresty et al. (1996)
reported an association between oral leukoplakia and two metabolites of the tobacco – specific nitrosoamine i.e. 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK). These two metabolites are 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) and [4-(methylnitrosamino)-1-(3-pyridyl) butyl]-beta-O-D-glucosiduronic acid (NNAL-Gluc). A significant association was found between the presence of leukoplakia and increasing levels of these metabolites. This study demonstrates chemical support for the role of smokeless tobacco as a cause of oral leukoplakia.

Warnakulasuriya (1995) reviewed four case-control studies that examined relative risk of oral leukoplakia in betel quid chewers. In one of the studies, chewing areca (in betel quid without tobacco) raised the odds ratio (OR) to 5 compared with non-chewers (OR= 1); adding tobacco to the quid further raised the relative risk by at least threefold compared with non-tobacco users. More recently, the results of a case-control study conducted in Taiwan, where areca is chewed without tobacco, found the odds ratio for developing leukoplakia was 17.43 (95% CI 1.94-156.27) for areca nut chewers. Furthermore, the authors also demonstrated that the cessation of areca chewing resulted in resolution of 62% of leukoplakias, suggesting that areca nut alone also has a significant etiological role in the development of leukoplakia.

**Oral Submucous Fibrosis:**

The ancient Indian Medical literature (Sushruta) described a condition “VIDARI” under mouth and throat diseases, in which progressive narrowing of mouth, depigmentation of oral mucosa and pain on taking food occurs (Mukherjee and Biswas, 1972). These are typical clinical features of oral submucous fibrosis (OSMF), the term coined by Joshi (1952). OSMF is an insidious chronic disease affecting any part of the oral cavity and sometimes the pharynx. Although occasionally preceded by and/or associated with vesicle formation, it is always associated with a juxta-epithelial inflammatory
reaction followed by a fibro-elastic change in the lamina propria, with epithelial atrophy leading to stiffness of oral mucosa and causing trismus and inability to eat (Hayes, 1985). The disease is characterized by burning sensation in mouth especially on eating spicy food. Vesicle formation, ulceration, excessive salivation and xerostomia may accompany the disease. The mucosa eventually becomes stiff, blanched and opaque and fibrotic bands appear usually involving buccal mucosa, soft palate, lips and tongue. OSMF is a chronic fibrotic disease of the oral cavity and oropharynx. It is predominantly seen in people in South Asian countries such as Bangladesh, Bhutan, India, Pakistan and Sri Lanka, or in South Asian immigrants to other parts of the world. It also occurs in people from Taiwan, China, Nepal, Thailand and Vietnam. It is extremely rare in white populations (Avon, 2004). This condition affects approximately 0.5% (5 million people) of the population in the Indian subcontinent and, following migration from this region, is now a health care problem in many parts of the world as reported by Canniff et al. (1986).

Three cases of oral submucous fibrosis among Chinese were also reported and were called as 'Idiopathic scleroderma of the mouth' by Su (1954). He mentioned that oral mucosa appeared dry, pale, atrophic and with marked trismus and suggested that it was due to high tannic acid content of betel nut, slaked lime and arecoline (areca nut alkaloid). Lee and Chin (1970) observed the effects of betel nut chewing on the buccal mucosa of 296 Indians and Malays in West Malaysia. They found varying degrees of epithelial atrophy, reduction of rete pegs, subepithelial inflammatory edema and fibrosis. A close association between OSMF and habituation to pan supari chewing was also reported by Dockrat and Shear (1970). Smith et al. (1975) found 0.49% prevalence of oral submucous fibrosis among the industrial workers in Gujarat, India. They observed that oral submucous fibrosis is more commonly associated with chewing of plain supari, or with combination of pan with chuna (slaked lime), kattha (paste of catechu), supari.
and tobacco than with other combinations. They also found association of oral submucous fibrosis with chilli intake.

Shiau and Kwan (1979) studied thirty-five Taiwanese patients with oral submucous fibrosis in the age range of 30-50 years and observed that most commonly, fibrotic changes were seen in buccal mucosa and palatal mucosa. The habits of areca nut chewing, tobacco chewing and consumption of hot spicy food were correlated with the occurrence of this disease. Among 60% of them were areca nut chewers and 51.4% smokers. The distribution of the mucosal involvement showed buccal mucosa as the site of maximum involvement (37.1%) followed by palate (29%), tongue (11.4%), floor of mouth (11.4%), gingiva (8.6%) and lip (2.9%), and 50% of the patients complained of having difficulty in opening of the mouth which varied from 10 to 30 mm. Gupta et al. (1980) demonstrated that the incidence of submucous fibrosis was nil among people with no chewing habit, whereas the incidence rate among the people who chewed areca nut along with or as an ingredient of pan was 35 per 1,00,000 people per year. Canniff et al. (1986) also analyzed forty-four patients with oral submucous fibrosis. All were areca nut chewers, of which some of them chewed along with additives as pan supari. Out of these, 68% were heavy chewers while 37% were moderate chewers. Among them 86% reported a burning sensation with hot or spicy food while 84% complained of limited mouth opening. Acute ulcerations and dry mouth was observed in six of these patients.

Bhonsle et al. (1987) studied oral submucous fibrosis in two districts of India. They observed a variation in the pattern of occurrence of OSMF. In Pune, Maharastra (India) OSMF affected more on the soft palate, uvula and retromolar region in contrast to patients in Ernakulam, Kerala (India) where the tongue, floor of the mouth and hard palate were more often involved. In addition, they observed that associated leukoplakia, oral cancer and petechiae were seen in these groups of patients. The age of patients differed
between these two places, Pune patients being younger than the other group. However both groups had pernicious habit of chewing tobacco. The patients of Ernakulam would spit out the juice macerated quid after chewing while the other group chewed for more time before swallowing the whole mixture. This could be the reason for the difference in sites of occurrence. A study was carried out among areca nut chewers in Taiwan. It was found that betel quid chewing is the main cause of OSMF and oral cancer. Lu et al. (1993) surveyed 2,442 junior high school students in Changhua country, Taiwan for the habit of areca nut chewing. Significantly more male students chewed areca nut than female students i.e. 9.2% versus 0.9%.

Babu et al. (1996) reported that habitual chewing of pan masala or gutkha is associated with earlier presentation of OSMF than betel quid use. Factors which may be responsible for these differences, are the tobacco content, the absence of betel leaf and its carotenes and the much higher dry weight of pan masala or gutkha as compared to betel quid i.e. pan. Gupta et al. (1997) selected a cohort of 12,212 villagers on the basis of their reported tobacco use in Ernakulam district of Kerala state. They followed annually over a period of 10 years for education on tobacco habits and the development of oral precancerous changes in house-to-house surveys. Analysis of incidence rates revealed that some lesions were almost solely associated with smoking habits, whereas oral submucous fibrosis and oral lichen planus-like lesion were solely associated with betel-quid chewing habits. Oral lichen planus and leukoplakia were associated with smoking as well as betel quid chewing habits. Gupta et al. (1998) undertook a study to determine whether there was an increase in the incidence of OSMF in the Bhavnagar district in Gujarat, India. The reported prevalence of OSMF in Bhavnagar district during 1967 was 0.16%. Among 5018 men who reported the use of tobacco or areca nut, 164 were diagnosed as suffering from OSMF. Areca nut was used mostly in the form of mawa, a mixture of tobacco, lime and areca nut and 10.9% of mawa users had SMF. The disease as well as areca nut use was about 85%
among lower (< 35 year) age group and concluded an increase in the prevalence of OSMF, especially in the lower age groups, directly attributable to the use of areca nut products.

Experimental evidence for the etiological role of the betel nut (areca catechu) in oral submucous fibrosis came from in vitro studies in which it was shown that ethanolic extracts of the nut stimulated collagen synthesis in human fibroblast (Caniff and Harvey, 1981). Meghji and colleagues (1982) investigated the effects of areca nut on the inhibition of collagenase and thereby aid in the deposition of excess collagen. They observed that areca nut extracts, purified tannins and catechin (the diphenol tannin precursor), all inhibited collagen lysis by both bacterial and mammalian collagenases in a dose-dependant manner. They concluded that tannin from the chewed areca nuts might enhance the development of fibrosis in oral submucous fibrosis by reducing the susceptibility to collagen degradation by collagenase. Harvey and associates (1986) suggested that the unnatural accumulation of collagen in the tissue of oral submucous fibrosis patient is due to fibroblast proliferation and stimulation of collagen synthesis by the alkaloids in the areca nut, as well as an inhibition of collagen degradation by the tannins and flavanoids which are also contained in the nuts. They further suggested that the fibroblast proliferation could also be aided by the juxta-epithelial inflammatory infiltrate of mononuclear leucocytes. Scutt and his colleagues (1983) studied the in vitro effects of areca nut alkaloids and GABA on fibroblasts and found that, the two compounds may alter collagen synthesis via a common pathway. Hydrolysis of arecoline to arecaidine appears to be the prerequisite for the stimulation of collagen synthesis by arecoline in fibroblasts.

Recent evidence suggests upregulation of the copper dependant extracellular enzyme lysyl oxidase by fibroblasts in OSMF is important, leading to excessive cross-linking and accumulation of collagen (Ma et al. 1995). Trivedi et al. (1997) examined the dry weight of copper in eight areca
nut products and the soluble copper in these samples products after extraction with water. The mean dry weight of copper was $302 \pm 92$ nmol/g (range 205-535 nmol/g), much higher than reported in other types of nuts commonly consumed as snacks in Britain. They suggested that there is an upregulation of the copper dependant extracellular enzyme lysyl oxidase which stimulates fibroblasts in oral submucous fibrosis, leading to excessive cross-linkage and accumulation of collagen. Anuradha and Devi (1995) observed that the patients with oral submucous fibrosis have low to normal serum concentration of copper and only 0.05% of areca-nut chewers in the Indian subcontinent develop OSMF without any evidence of liver cirrhosis.

The most serious aspect of OSMF is its high potential for development of cancer; the relative risk being 400 times (Gupta, 1999). Murti et al. (1985) assessed the malignant transformation rate in 66 patients with oral submucous fibrosis at the end of 17 years of observation with the corresponding median observation period of 10 years. Oral cancer developed in 5 patients, giving a malignant transformation rate of 7.6%. All 5 patients in whom cancer developed were women who had the habit of chewing tobacco and areca nut with betel leaves and lime. Oral cancer developed 3-16 years after the diagnosis of oral submucous fibrosis. Thus, the data available suggests that OSMF is a precancerous lesion. The possible precancerous nature of OSMF was first described by Paymaster (1956) who observed occurrence of squamous cell carcinoma in one-third of patients with OSMF. Subsequent studies reported that the incidence of carcinoma varies in OSMF from 2 to 30% (McGurk and Craig, 1984). Awang (1986) investigated the pharmacology of betel nut (areca catechu) in relation to oral submucous fibrosis. They found that boiling the nut, commonly used for softening before chewing removed the majority of alkaloids. The variations in nut alkaloids and tannin content were probably due to plant variability and different procedures for the preparation of the areca nut for consumption. These variations in the pharmacologically active constituents of the betel nut may contribute to the
regional difference in the incidence of the disease. Thus, it has been established that betel nuts (with or without paste of crude slaked lime and spices and/or tobacco) might play a role in the initiation and pathogenesis of oral submucous fibrosis. However, only a fraction of the betel nut chewers develop OSMF suggesting genetic susceptibility or lack of antifibrotic activity in OSMF cases. Canniff et al. (1985) demonstrated a genetic predisposition to the disease, involving raised frequencies of HLA antigens A\textsubscript{10}, DR\textsubscript{3}. The histopathological and clinical features of the oral mucosa gave the suggestion of an autoimmune basis for oral submucous fibrosis. The results supported that OSMF is a chronic autoimmune disease, initiated by constituents of betel nut, and occurring in genetically susceptible individuals.

**Oral cancer:**

Oral cancer is one of the common cancers in the world. Fifty eight percent of the 390,000 oral and oro-pharyngeal cancers estimated to occur annually in the world occur in South and South-East Asia (WHO, 2003). In India, Bangladesh, Pakistan and Sri Lanka it is the most common cancer and accounts for about a third of all cancers. Recently, oro-pharyngeal cancer has been reported to be fifth most common cancers of humans (Jeng et al. 2001). Experimental studies reveal that betel nut extract might have mutagenic and genotoxic effects in addition to inducing pre-neoplastic as well as neoplastic lesions in experimental animals with varying degree of confirmation. Wynder et al. (1957) conducted a study of 659 cases of lip and oro-pharyngeal cancers and found that 17% of cases chewed tobacco in contrast to 8% controls, indicating a moderate association between tobacco chewing and these cancers. Several descriptive studies show that the frequency of chewing habits among patients with oral cancer is so high that, despite the absence of controls, tobacco usage could be incriminated as a causative factor for oral cancer. Paymaster (1962) reported that 81% of 4212 oral cancer patients used tobacco and of these 36% were chewers, 23% smokers, and 22% practiced both chewing and smoking. Several other investigators have made
similar observations from India (Singh and Von Essen, 1966; Srivastava and Sharma, 1968; Gandagule and Agarwal, 1969; Samuel et al. 1969) and in Taiwan (Chang, 1964). The site of origin of oral cancer corresponding to the area of placement of tobacco quid (Hirayama, 1966) is also strong evidence in favour of the role of betel-tobacco chewing in oral cancer. Several investigators observed the occurrence of oral cancer at the site of placement of quid also (Sorger and Myrden, 1960; Kraus and Perez-Mesa, 1966; Axell et al. 1978; Sundstrom et al. 1982; McGuirt, 1983). Goud et al. (1990) suggested that this occurs almost always on the side of the mouth where tobacco quid (particularly night quid) is kept. The dose-response relationship provides the trend of association between extent of exposure of etiological agent and risk of oral cancer. The relative risk of oral cancer in the study by Hirayama (1966) was 8.4 when frequency of chewing was less than 2 per day. It increased to 17.6 when frequency rose to 6 or more per day and it was 63 when quid was retained in the mouth while asleep.

Cross-sectional studies conducted by Mehta et al. (1969 and 1972) provide the prevalence of oral cancer among people with or without tobacco habits. In these studies in over 1,50,000 individuals, although there were a substantial number of non-habitues, 14 of 38 oral cancers occurred in people who were solely betel-tobacco chewers, 24 among other tobacco chewers, and none among non-chewers. In the most extensive study of its kind, Gupta et al. (1980) followed up 30,000 individuals over a 10-year period in three areas of India. The betel-tobacco chewing habit was common in Ernakulam, and the annual age adjusted incidence of oral cancer was 23 per 100,000 among betel-tobacco chewers. In another study the incidence of oral cancer among persons with smoking and pan supari chewing habit was 0.09% as against 0.02% among non-users (Smith et al. 1975).

Gupta (1982) showed from a literature review that the relative risk of chewing betel quid without tobacco for oral cancer was either insignificant or
significantly lower than the relative risk for betel quid with tobacco. While many studies point out that tobacco is the principal etiological agent for oral cancer. Atkinson et al. (1964, 1982) suggested that areca nut and lime had a definite carcinogenic effect even when chewed without tobacco; this conclusion was based on their observations in Papua New Guinea, where oral cancer was very common, and people chewed betel leaf, areca nut and lime without addition of tobacco. Other investigators also made similar suggestion subsequently from Papua New Guinea (Jamrozik, 1985; MacLennan et al. 1985). The habit of chewing betel quid with or without tobacco has often been mentioned in the literature as betel chewing or betel nut chewing and this has further contributed to the confusion as to whether the principal carcinogenic effect is due to tobacco or other ingredients. It needs to be 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 people were chewers and only 3% did not include tobacco in the quid (Mehta et al. 1971,1972).

*Micronucleus test as biological marker of genotoxic exposure:*

The data available suggests that the smokeless tobacco chewing has been associated with various oral diseases including oral cancer. Thus, there is need to assess the changes in oral cavity, which may be useful for the early detection of oral diseases and might act as biomarkers. There are reports of smokeless tobacco chewing association with the premalignant conditions such as oral leukoplakia, oral submucous fibrosis or oral cancer. Some reports also implicated use of areca nut alone in these changes with varying degrees of positivity. Keeping in view of the wide spread habits of chewing betel nut with or without tobacco around the world, it is necessary to look into the biomarkers. Studying the oral mucosa for any changes will be directly correlated with the chewing habits as oral mucosa is in direct contact with the chewing material while chewing. Thus, in this study, micronuclei in the buccal
mucosa cells were observed, which could be correlated with the habits and 
might serve as potential genotoxic biomarkers of the chewing material.

Mutations in cells are known to play an important role in induction of 
cancer. Short-term assays like the micronucleus test (MN), chromosome 
aberration (CA) and sister chromatid exchange (SCE) analysis are sensitive 
and well-established cytogenetic markers of DNA damage. The micronucleus 
test is an indirect and sensitive measure of chromosomal breakage or 
missegregation and has received increased attention in recent years as a 
sensitive biologic marker of genotoxic exposure. The MN test permits a non-
invasive assessment of human tissues as well as an opportunity for repeated 
samplings, which appears to be ethically acceptable. Micronuclei are small, 
round to oval shaped bodies found within the cytoplasm but outside the main 
nucleus. Because they resemble the main nucleus in texture, staining 
properties and contain DNA, they can be easily detected as markers of 
missegregated chromatin. Micronucleus assay is a potential method to screen 
human cells rapidly and cost effectively for genetic damage. Exfoliated cells 
hold strong potential as a tool for biomonitoring human populations. Stich et 
al. (1982 a, b) and Stich et al. (1983) used this assay to epithelial cells of 
buccal mucosa so that target tissues or cells could be studied. Stich et al. 
(1982 a, b) used the micronucleus assay to exfoliated oral epithelial cells of 
khaini tobacco chewers of Bihar, India and reported an elevated frequency of 
MN in all the individuals examined. Dave et al. (1991) used three cytogenic 
end points and two tissues were employed to assess possible DNA damage 
among pan masala chewers and controls. Sister chromatid exchange and 
chromosome aberrations were estimated in peripheral blood lymphocytes, 
tissues indirectly exposed to substances. The frequency of MN cells was 
scored in the tissue directly in contact with pan masala, i.e. the exfoliated 
buccal mucosal cells. All the three cytogenic end points demonstrated 
statistically significant increase among the pan masala consumers as 
compared to non-consuming controls. Earlier, Shirname et al. (1984) reported
that an aqueous extract of betel quid without tobacco was not mutagenic to Chinese hamster V 79 cells with or without S9 and did not induce micronuclei in bone marrow cells. However, an aqueous extract of betel quid with tobacco and aqueous extract of areca nut alone induced micronuclei in bone marrow cells of Swiss albino mice.

Livingston et al. (1990) reported that unlike the case with cigarette smokers, peripheral lymphocytes sister chromatid exchange frequency was not affected by exposure to smokeless tobacco. The oral cytology data, however, support an interpretation of exposure dependent nuclear alterations, including micronuclei in the oral epithelium associated with the use of smokeless tobacco. Further, Livingstone et al. (1990) reported (unpublished data) studies on lymphocyte micronuclei which show that this test is sensitive to X-irradiation as low as 5 rad, cytostatic drug concentration as low as 5 ng/ml, and a polycyclic aromatic hydrocarbon (benzo(a)pyrene) as low as 1 μg/ml. Two of these environmental agents ( radiation-emitting polonium and benzo(a) pyrene) are known to be present in smokeless tobacco (US Dept Health and Human Service, 1986). The reliability of MN assay as a screening test for somatic cell genetic damage is strongly supported by several observations. Thus, MN was studied in the representative numbers of subjects from both chewers and non-chewers.

**Serum immunoglobulins in oral mucosal lesions:**

Immunoglobulins are glycoprotein molecules which are produced by plasma cells in response to an immunogen and which function as antibodies. The immunoglobulins derive their name from the finding that when antibody-containing serum is place in an electrical field the antibodies migrated with the globular proteins. Immunoglobulins bind specifically to one or a few closely related antigens. The primary function of antibodies is protection of the host. However, often the binding of an antibody to an antigen has no direct biological effect. Rather, the significant biological effects are a
consequence of secondary "effector functions" of antibodies. The immunoglobulins mediate a variety of these effector functions e.g. complement fixation, opsonization, etc. Usually the ability to carry out a particular effector function requires that the antibody bind to its antigen. Not every immunoglobulin will mediate all effector functions. The immunoglobulins can be divided into 5 different classes based on differences in the amino acid sequences in the constant region of the heavy chains. All immunoglobulins within a given class will have very similar heavy chain constant regions. These differences can be detected by sequence studies or more commonly by serological means.

- **IgG (Gamma heavy chains):** IgG is the most versatile immunoglobulin and the major immunoglobulin in serum - 75% of serum immunoglobulin is IgG. It is the only class of immunoglobulin that crosses the placenta. It fixes complement and enhances phagocytosis through opsonization.

- **IgA (Alpha heavy chains):** IgA is the second most common serum immunoglobulin. It is the major class of immunoglobulin in secretions - tears, saliva, colostrum, mucous. Since it is found in secretions, secretory IgA is important in local (mucosal) immunity. It can bind to some cells - polymorphonuclear cells and some lymphocytes.

- **IgM (Mu heavy chains):** IgM is the third most common serum immunoglobulin. It is the first immunoglobulin to be made by the fetus and the first immunoglobulin to be made by a virgin B cells when they are stimulated by antigen. As a consequence of its pentameric structure, IgM is a good complement fixing immunoglobulin and also a good agglutinating immunoglobulin. Surface immunoglobulin present on B cells helps them to differentiate into antibody secreting plasma cells.
• IgD (Delta heavy chains): It is found in low levels in serum; its role in serum uncertain. IgD is primarily found on B cell surfaces where it functions as a receptor for antigen.

• IgE (Epsilon heavy chains): IgE is the least common serum immunoglobulin. It is primarily involved in allergic reactions. Binding of the allergen to the IgE on the cells results in the release of various pharmacological mediators that result in allergic symptoms. It also plays a role in parasitic helminthic diseases.

It was hypothesized that serum levels of immunoglobulins may play an important role in the pathogenesis of oral mucosal diseases, or reflect clinical changes in these conditions (Sisting et al. 2002). Moszczynki et al. (2001) reported that the suppressive effect of tobacco smoke on human immunity was seen as decreased serum concentrations of immunoglobulins and lysozyme, especially in men smoking more than 10 years, decreased (CD16+) NK- cells absolute number and elevated population of (CD8+) T-cytotoxic lymphocytes. Earlier Andersen et al. (1982) also reported that tobacco smoking might suppress the humoral immune response. They further reported that serum IgG and IgA levels were higher in nonsmokers than smokers. However mean serum IgM values were not significantly different between two groups. Moszezynski et al. (1989) found that both tobacco smoke and organic solvents, when acting separately, diminish the IgA and IgG level in the serum. Additionally, in the smokers the lowering of the IgM level occurred. In smokers occupationally exposed to benzene and its homologues the decrease in the IgA, IgG and IgM level in the serum was more significant than in those exposed to either tobacco smoke or organic solvents.

Shah et al. (1994) studied the saliva immunoglobulin levels in OSMF cases. They reported that saliva IgA, IgG and IgM levels were raised significantly in OSMF cases compared to control. Further, Soto (1991)
reported that the secretory immunoglobulin A (SIgA) in the whole saliva did not show any major age or sex related difference. However, patients with leukoplakia, lichen planus and carcinoma in the oral cavity showed higher levels of SIgA. The elevated levels of SIgA in the saliva of these patients suggested that some local changes in immunological competence might occur due to exposure to the antigen in the oral cavity.

McMillan et al. (1997) studied the effect of low to moderate levels of smoking and alcohol consumption on serum immunoglobulin concentrations and found that low to moderate consumption of alcohol was associated with a decrease in IgG and IgM median concentrations in contrast to an increase in IgA median concentrations. The decrease in IgM and especially IgG median concentrations appeared to be related to the smoking habits of the subjects. They concluded that low levels of alcohol consumption and cigarette smoking influence IgG, IgM and IgA serum concentrations. Very recently Gyllen et al. (2004) reported that tobacco smokers have lower serum levels of immunoglobulin IgG, mainly due to lower levels of IgG2, than non-smokers. The components in tobacco smoke responsible for this effect is unknown, but animal studies have implicated nicotine as a major contributor to the immunologic effects of smoking. They further reported that smokeless tobacco or nicotine replacement therapy has no effect on serum immunoglobulin levels. There is scanty data available on the serum immunoglobulin levels among chewers of smokeless tobacco and areca nut. Thus, serum immunoglobulin (IgG, IgA and IgM) levels were measured using immunodiffusion method in representative number of samples of chewers and nonchewers.

**Role of copper and zinc metals on oral health and disease:**

Metals have always been an intrinsic component of earth’s crust. Humans have been in close contact with metals almost since the beginning of our existence. In fact, one cannot even think of human evolution without
considering the valuable role played by the metals in development of mankind. Some metals are essential for human nutrition; others are found as contaminants in foodstuffs. One feature of the normal human diet frequently found is the simultaneous presence of both, essential and toxic metals. Recent years have seen advances in the knowledge of the significance of trace elements in human health and diseases. The global health and social costs of nutritional disorders are apparent and documented. Micronutrients have an enormous impact on health and productivity due to primary effects and their co-factors in a variety of conditions including oral cavity, gastrointestinal tract and other infections, low birth-weight, psychomotor and cognitive development etc. Factors important in the risk-evaluation analysis of metals are their pharmacokinetics, interactions among them and with other major components of the dietary habits of different populations and the regional distribution of metals. Areca nut chewers are also exposed to some amount of toxic/essential metals during chewing as well as through environmental and occupational exposure. The toxic effects of certain metals in different animal systems are well documented. The areca nut is reported to contain appreciable amount of copper as compared to other nuts commonly consumed as snacks (Trivedy et al. 1997). Some of the metals such as cadmium, lead etc are present as contaminants in the chewing products like pan masala (NIOH, 1989).

Metals are not biodegradable, have long biological half-lives and have the potential for accumulation in the different body organs leading to unwanted side effects. The advent of modern techniques that were developed during last 20 years such as atomic absorption spectrometry, microwave excitation emission and atomic fluorescence has made quantitation of even minute amounts of these micronutrients possible. Recent researches implicated copper in several fibrotic conditions (Britton, 1996). Copper is an essential trace element for the function of several key enzymes involved in human metabolism (Linder et al. 1996). These include cytochrome-c oxidase,
superoxide dismutase, metallothionein and lysyl oxidase. Abnormalities in copper absorption, metabolism and excretion can lead to deposition of copper in several body sites. These include genetic disorders like Wilson's disease (Taylor, 1996) or environmental contamination leading to copper accumulation in Indian childhood cirrhosis and pulmonary fibrosis (Baker, 1995). Serum copper concentrations and ceruloplasmin often remain low or are normal in Wilson's disease but increase with progression of the disease. While in Indian childhood cirrhosis copper levels are raised in the body fluids (Trivedy et al. 1999). Earlier they demonstrated that areca products contain a high level of copper (mean 302 nmol/g) when compared to other commonly eaten nuts (22-173 nmol/g) and that soluble copper is released into whole mouth saliva following chewing areca for 5-30 minutes (Trivedy et al. 1997). Further, copper might also have a significant role in the development of OSMF, as there is a report that supports the hypothesis of copper as a factor in OSMF (Trivedy, 1999). Thus there is a need to have the base line data on copper in the saliva and serum of chewers and non-chewers.

Heavy metals are incorporated through food ingestion and inhalation. Zinc, copper, manganese, chromium and selenium are the essential trace metals required for various physiological functions. Zinc is one of the most abundant nutritionally essential elements in the human body. It is found in all body tissues with 85% of the whole body zinc in muscle and bone, 11% in the skin and the liver and the remaining in all the other tissues. It has been shown to be essential to the structure and function of a large number of macromolecules and over 300 enzymic reactions. (Tapiero and Tew, 2003). Zinc deficiency has been associated with adverse effects in humans and animals, however, over exposures to zinc also have been associated with toxic effects. (ATSDR, 1994). The regeneration of the oral mucosa is zinc and vitamin A dependant. Hyperkeratosis, a disturbed immune system and a high incidence of oral malignancies have been reported in cases of deficiency by Kleier et al. (1998). They observed significant lower level of zinc in
leukoplakia and oral cancer patients compared with the control group. Jayadeep et al. (1997) also reported a significant decreased zinc level only in male patients with leukoplakia and squamous cell carcinoma. However, they noted the significantly increased level of copper in oral leukoplakia and cancer patients in both sexes. But, earlier Varghese et al. (1987) noted a significant reduction in the serum copper and zinc levels in both OSMF and oral cancer. Yoshida (1989) studied the effects of zinc deficiency on rats' oral mucosa and observed hyperkeratosis in buccal mucosa, ventral surface and interpapillary mucosa of the tongue in rats fed with zinc low diet. He also suggested that zinc deficiency might have a serious consequence on the oral mucosa in its tolerance.

The habit of chewing areca nut has existed on the Indian subcontinent and throughout the far East for thousands of years, and its popularity rests on the presence of pyridine alkaloids arecoline, arecaidine, guavacine and guavacholine in areca nut. This group of psychotropes is one of the five families of pleasure poisons, which account for global drug addiction, the other members being nicotine, cocaine, the opium alkaloids and lysergic acid and its derivatives. The areca alkaloid arecoline is hydrolyzed to arecaidine in vivo, which is a competitive inhibitor of gamma-aminobutyric acid (GABA) uptake in the brain, an activity which may explain the psychotropic attraction of the betel nut habit. (Johnston et al. 1975). Hydrolysis of arecoline to arecaidine during chewing of the nut is facilitated by the time-honored practice of including slaked lime with the quid (Boyland, 1968).

It has been suggested that smokeless tobacco, or some of its components, may contribute to degenerative changes in human salivary glands (Peterson and Pindborg, 1973; Hirsh, 1982). However, the data are inconclusive, and it has been suggested that salivary gland fibrosis and degenerative changes may be associated only with particular tobacco brands, and are thus not a generalized reaction to all tobaccos. In view of scanty data,
salivary gland data should be interpreted with caution and it should be remembered that any reduction in salivary flow would result in a decrease in protective factors for the oral epithelium, as well as for the exposed crown and root surfaces (US Dept. of Health & Human Services, 1986).

Keeping in view of the above, further studies with sufficient power and adequate control of confounding factors are required to elucidate the role of areca nut use in oral disease. Studies would benefit from improved validation, trend information, and consideration of individual brands. More information is urgently required on the potential health effects of areca nut use other than cancer, particularly oral lesions and various systemic effects. A difficulty in many countries has been obtaining sufficient numbers of areca nut users to enable precise estimates of risk. Populations with high use of these products need to be identified and followed over time. There is some evidence that cigarette smokers have quit habit and become smokeless tobacco users and some are promoting this as a method of "tobacco harm reduction". Long term follow up of populations of smokers who either quit tobacco use or become chewers should be established to compare the differences in a range of health outcomes between these two groups. Only further well designed epidemiological studies with adequate sample sizes will be able to resolve these controversies.

Thus, the convergence of epidemiological and laboratory evidence supports a relationship between the use of areca nut and pan masala (with tobacco) and local and systemic disorders ranging from mere staining of teeth to development of life threatening cancerous lesions. The results of these studies clearly indicate the need to intensify the work towards development of surveillance, monitoring and evaluation systems and develop programme directed towards control of this new epidemic. Further, more planned epidemiological studies are needed to find out the role of various chewing habits and oral disease.