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Toxic Effects of Betel Quid

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ABSTRACT Oral cancer is most common cancer in males and third most common in females, the main causative agent being use of chewing betel quid (BQ). Areca nut, Cathechu, Slaked lime are major components of Betel quid. Nitrosamines formed from alkaloids in betel nut during betel quid chewing may be implicated in the etiology of oral cancer. Reactive oxygen species are generated due to slaked lime which is also present in betel quid. Micronuclei (MN) have been proposed as a good biomarker to assess cytogenetic damage. Percentage of MN formation has been observed in pre cancerous lesions of the oral cavity of betel quid chewers. In this present study cases were screened from Department of E.N.T. & Oral and Maxillofacial surgery of Ramakrishna Mission Seva Pratishthan hospital, Kolkata and different areas of West Bengal. Some of them had more than one addiction and some have no addiction and complications. Hemogram reports are normal but mitotic index and micronuclei higher in oral cancer cases than normal.

INTRODUCTION

Betel chewing is a popular habit in Asia. "Betel quid chewer's oral cancer" is one of the most common malignancies in South and South east Asian countries. Oral premalignancies are also very common in betel quid chewers and about 10% of these undergo malignant transformation. The main ingredients used in quid are areca nut, catechu, betel leaf, slaked lime. The major areca nut alkaloids are arecoline, arecaidine, arecolidine, guacine. Arecoline are the most abundant alkaloid. Secondary and tertiary amines which are present in the areca nut undergo nitrosation and give rise to N- nitrosamines. The nitrosation of arecoline may produce a variety of betel quid specific nitrosamines (BQSN). The BQSN interact with DNA, proteins or other targets forming adducts to exert its carcinogenic activity.

Oral cancer is the fifth most common cancer worldwide (Parkin et al. 1993). A 2- to 3-fold increase in mortality has been recorded in eastern and central European countries in recent decades (Coleman et al. 1993). It has been estimated that, worldwide, ~600000000 people chew areca nut (Nelson and Heischober 1999).

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DESCRIPTION OF BETEL QUID

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* (areca nut,slaked lime, catechu, condiments and tobacco), *mainpuri* (tobacco, slaked lime, areca nut, camphor and cloves), *mawa* (areca nut, tobacco and slaked lime), *khaini* (tobacco and slaked lime), *gutka* (an industrially manufactured food item) and other smokeless tobaccos (*mishri, gudhaku, bajjar* etc.). A causal association between tobacco and betel quid (BQ) chewing habits and oral mucosal diseases such as leukoplakia, oral submucous fibrosis. Of the 390 000 oral and oropharyngeal cancers estimated to occur annually world wide, 58% occur in south and south-east Asia. In India there are 75 000-80 000 new cases of oral cancer each year and the incidence rates of cancers of the oral cavity in both males and females in all urban cancer registries are among the highest in the world. In India were estimated to be 12.8 in men and 7.5 in women (Douglass et al .1984; Ferlay et al. 2001). Oral cancer occurs more commonly among men than women depending upon the extent and type of tobacco habits prevalent. Betel quid chewing is the major risk factor for buccal mucosal and gingival cancer. For the tongue cancer most frequent in Western countries smoking is the major risk factor. Chewing of BQ and areca nut is an ancient custom in several parts of south-east Asia, the south Pacific islands and Taiwan. A ceremonial gift of dried tobacco leaves given to Columbus by Native Americans in 1492 led to the intro-

duction of tobacco into the rest of the world. It arrived in India in the 16th century; a sample was presented to the Emperor Akbar, who patronized smoking, rapidly spreading the habit in the sub-continent. The BQ is a mixture of areca nut (Areca catechu), catechu (Acacia catechu) and slaked lime (calcium oxide and calcium hydroxide) wrapped in a betel leaf (Piper betle) . Betel nut is composed of 11.4 – 26.0% tannins, 0.15 -0.67% alkaloid,1.3 -17% fat ,0.13 -2.35% phosphorus , 1.5 -11.6% iron (Raghavan and Barua 1958). The major areca nut alkaloids are arecoline, arecaidine, arecolidine, guvacoline and guacine (IARC Monograph 1985). Arecoline (1,2,4,5,-tetrahydro-1-methylpyridinecarboxylic acid; molecular weight 155.19) is the most abundant alkaloid of areca. These alkaloids undergo nitrosation and give rise to N-nitrosamines (Hoffmann et al. 1994). Betel nut used as an anthelmintic in humans and animals (Arjungi 1976). It is expected that over 250 millions inviduals addict the betel chewing habit (Stich et al. 1983).

ORAL CANCER AND PRECANCEROUS CONDITION

Oral cancer, a malignancy of the lip, mouth or tongue, is predominantly a squamous cell carcinoma. Chewers of BQ with or without tobacco often develop clinically visible whitish (leukoplakia) or reddish (erythroplakia) lesions and/or stiffening of the oral mucosa and oral submucous fibrosis (OSF). Leukoplakia is one of the commonest lesions in betel quid chewers. The WHO has classified these into two groups, homogeneous and non-homogeneous. Among no homogeneous leukoplakias, nodular leukoplakia tends to show the highest rate of malignant transformation. The relative risk compared with individuals with tobacco habits but without any precancerous oral lesion was also found to be the highest for nodular leukoplakia (Gupta et al. 1989). Oral sub mucous fibrosis (OSMF) is a chronic condition characterized by mucosal rigidity of varying intensity due to fibro elastic transformation of the juxtaepithelial layer (Murti et al. 1995). OSMF is a high-risk precancerous condition (Pindborg et al. 1984) with a malignant transformation rate of about 7.6% (Murti et al. 1985). Areca nut chewing could be one of the most important etiologic factors in OSMF (Sinor et al. 1990).All these well-established precancerous lesions are easily diagnosed and present an important indicator of oral cancer risk. The malignant transformation of non homogeneous lesions involving erythroplakia and nodular leukoplakia is particularly high, reportedly ranging from 15 to 40% depending upon the time period (Sankaranarayanan et al. 1997). OSF is predominantly caused by the use of areca nut (Murti et al. 1995). Marked by stiffening of the oral mucosa and development of fibrous bands, loss of elasticity of the mucosa results in a progressive restriction of mouth opening. Affected users experience a burning sensation of the oral mucosa, occasional mucosal ulceration, a peculiar marble-like blanching of themucosa and palpable fibrous bands of the buccal mucosa, softpalate and lips. Symptoms of cancer appeared at an early age in youngsters (Babu et al. 1996b). Oesophageal subepithelial fibrosis, an extension of oral submucosal fibrosis, was seen more frequently in patients who had consumed *pan masala*, *gutka*, areca nut, tobacco or a combination of some or all of these, with or without betel leaf, (Misra et al.1998). *Mawa*, a preparation similar to *gutkha*, containing tobacco, lime and areca nut slivers, has also been linked to OSF, oral cancer and oesophageal cancer. A study carried out in the Bhavnagar district in India, where chewing of *mawa* has mushroomed in recent years, showed a corresponding increase in OSF (Gupta et al. 1998).

CARCINOGENS IN BQ (BETEL QUID)

Several carcinogens are derived from tobacco but also from areca nut (Hoffmann et al. 1994). Chewing of tobacco with BQ results in high exposure to carcinogenic tobacco-specific nitrosamines (TSNAs), to ~1000 mg/day (Nair et al. 1999). The carcinogenic TSNAs N'-nitrosonornicotine (NNN), 4-(N-methyl-N-nitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and N-nitrosoanabasine (NAB),as well as the volatile nitrosamines N-nitrosodimethylamine and N-nitrosodiethylamine, have been detected in the saliva of chewers of BQ with tobacco (Wenke et al. 1984; Nair et al.1985; Bhide et al. 1986; Nair et al.1987a). TSNAs undergo metabolic activation by cytochrome P450s and other enzymes. NNK, a major carcinogenic TSNA, is activated by either methylene hydroxylation to generate an intermediate that decomposes to a

DNA-methylating agent,resulting in the formation of 7-methylguanine, O^6-methylguanine, (O^6-MeG) and O^4-methylthymidine in DNA or via methyl hydroxylation to form bulky pyridyloxobutyl DNA adducts.NNK is also converted metabolically to 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol, which can also be activated by a-hydroxylation to yield methyl and pyridylhydroxybutyl adducts in DNA (Hecht 2003). 2'-Hydroxylation of NNN, another important TSNA, can give rise to the same intermediate as is formed by methyl hydroxylation of NNK, resulting in pyridyloxobutylation of DNA. The areca nut-specific nitrosamines (ASNAs) N-nitrosoguvacoline(NG) (Wenke et al. 1984; Nair et al. 1985;Stich et al.1986; Nair et al. 1987a) and the carcinogenic 3-(methyl-N-nitrosamino) propionitrile (MNPN). Prokopczyk et al. (1987) were also detected in the saliva of chewers of BQ without tobacco. Nitrosation of BQ with nitrate and thiocyanate in vitro at neutral pH resulted in the formation of NG (Nair et al. 1985). The highest levels of an ASNA (NG) were found in the sediment of saliva collected from Taiwanese BQ chewers (Stich et al. 1986), whereas the highest levels of TSNAs have been found in saliva samples collected in India (Bhide et al.1986).

FORMATION OF *N*- NITROSO COMPOUNDS IN THE ORAL CAVITY

Volatile nitrosamines and tobacco-specific nitrosamines in the saliva of chewers are derived from leached-out preformed nitrosamines present in tobacco, but can also be formed endogenously from abundant precursors during chewing.Secondary and tertiary amines present in areca nut and tobacco can be nitrosated during BQ chewing when they react with available nitrite in the presence of catalysts such as thiocyanate (Nair et al. 1985; Nair et al. 1987a). The enhanced nitrosation in subjects with poor oral hygiene may be due to greater conversion of nitrate to nitrite and bacterial enzyme-mediated formation of nitrosamines or both (Calmels et al. 1996; Ziebarth et al. 1997). Elevated levels of nitrite and nitrate reductase activity have been reported in the saliva of Indian chewers of BQ with tobacco (Murdia et al. 1982). There is increased nitric oxide and nitrite formation in subjects during deposition of dental plaque (Carossa et al. 2001). Thus, in view of the availability of nitrosatable amines from areca nut and tobacco, increased formation of nitrosamines might be expected in the oral cavity of BQ, tobacco, pan masala and gutkha chewers. The acidic pH of the stomach would favour the nitrosation of secondary and tertiary amines in the quid.

REACTIVE OXYGEN SPECIES

Reactive oxygen species (ROS), implicated in multistage carcinogenesis, are generated in substantial amounts in the oral cavity during chewing (Nair et al.1987b; Nair et al. 1992; Nair et al. 1995) first demonstrated that aqueous extracts of areca nut and catechu were capable of generating superoxide anion and hydrogen peroxide at pH > 9.5. The areca nut-induced production of ROS was enhanced by $Fe^{2}+$, $Fe^{3}+$ and $Cu^{2}+$, but inhibited by $Mn^{2}+$. The presence of $Ca(OH)_2$ in slaked lime leads to alkaline conditions in the oral cavity, favouring ROS generation. Calcium hydroxide content of lime in the presence of areca nut is a major factor responsible for the formation of ROS which cause oxidative damage in the DNA of buccal mucosa cells of BQ chewers. Decreasing the slaked lime content of BQ should therefore reduce its toxicity. Hydroxyl radicals (OH´) were shown to be generated in vitro using L-phenylalanine as substrate together with some ingredients of BQ and pan masala. Therefore, the formation of *o* and *m*-tyrosine from L-phenylalanine can be measured as a marker of OH´ generation. Both *o*- and *m*-tyrosine were formed in vitro in the presence of extracts of areca nut and or catechu, transition metal ions ($Cu^{2}+$ and $Fe^{2}+$) and alkaline pH (slaked lime or sodium carbonate). OH´ are formed in the oral cavity of BQ chewers and probably implicated in the genetic damage observed in oral mucosal cells of chewers. By the same method, OH´ formation was monitored in Taiwanese subjects chewing tender areca nut and lime with either Piper betel or betel leaf (Chen et al. 2002). Levels of *o*- and *m*-tyrosine were increased but were lower than those detected in Indian chewers, perhaps due to differences in the BQ ingredients. Superoxide anion production, assayed by cytochrome C reduction and lipid peroxidation by formation of thiobarbituric acid-reactive substances, was demonstrated in normal human oral keratinocytes following exposure to commercially available gutkha and pan masala (Bagchi et al. 2002).

GENOTOXICITY AND MUTAGENICITY OF *PAN MASALA*

The areca nut, the major constituent of pan masala is responsible for mutagenic, clastogenic and carcinogenic properties (Jeng et al. 2001). Areca nut contains 5 - 40% polyphenols and several alkaloids including arecoline, arecaidine, guvacine and guvacoline. Arecoline, the most important areca nut alkaloid, is present at 1% of the dry weight and has been shown to be genotoxic (Dave et al.1992a). Exposure to aqueous areca nut extract induced mitotic gene conversion at pH > 10 (Rosinet al. 2002). Recently, areca nut chewing has been classified as carcinogenic to humans (IARC 2004). Aqueous extracts of both *pan masala* and *gutkha* induced chromosomal aberrations and micronucleated cells in Chinese hamster ovary cells in the presence or absence of an exogenous metabolic system, although metabolic activation markedly inhibited the chromosome damaging effect, implicating the presence of direct-acting mutagens (Dave et al.1991). Catechu, another constituent of *pan masala*, has mutagenic (Stich et al. 1983) and clastogenic activity (Giri et al. 1988). Lime is known to cause irritation and hyperplasia of the oral mucosa (Dunham et al. 1966).

GENOTOXICITY IN HUMANS

Chromosome breaks have been reported in oral exfoliated cells in chewers of BQ with or without tobacco. Micronucleus formation has been observed in precancerous lesions of the oral cavity of chewers (Nair et al. 1991). In vitro studies with cultured fibroblasts have shown that areca nut alkaloids such as arecoline and its hydrolysed product arecaidine stimulate proliferation and collagen synthesis in a dose-dependent manner (Canniff and Harvey 1981; Harvey et al. 1986), higher concentrations being cytotoxic (van Wyk et al. 1994; Jeng et al. 1996).

ARECA NUT AND ORAL SUBMUCOUS FIBROSIS (OSF)

Flavonoids, catechins and tannins in areca nuts cause collagen fibres to crosslink, making them less susceptible to collagenase (Scutt et al. 1987). This can cause increased fibrosis due to increased collagen production and decreased collagen breakdown. OSF is irreversible and persists even after cessation of the chewing habit, suggesting that components of the areca nut initiate OSF and then affect gene expression in the fibroblasts, which then produce greater amounts of normal collagen (Meghji et al.1987; de Waal et al.1997). In OSF patients with a habit of chewing areca nut or *pan masala*, a significant increase in total serum protein was observed with lower levels of ascorbate and iron, which are used in collagen synthesis. The total tissue collagen content increased significantly in patients with advanced disease and with progression of the disease, leading to hypomobility of the tongue, lips, cheeks, soft palate and faucial pillars (Anuradha and Devi 1993). It has been suggested that metabolic activation may involve the cytochrome p450 system (Sundqvist et al.1991; Wary and Sharan 1991). The nitrosation of arecoline may produce a variety of betel quid-specific nitrosoamines (BQSN). The BQSN interact with DNA, proteins or other targets forming adducts to exert its carcinogenic activity. Slaked lime is also included in betel quid. It causes inflammation in the submucosal area and Nair et al. have reported that the calcium hydroxide content of lime in the presence of the areca nut is primarily responsible for the formation of reactive oxygen species that might cause oxidative damage in the DNA of buccal mucosa cells of betel quid chewers (Nair et al. 1990).

INCIDENCES AND ETIOLOGY OF ORAL CANCER

Oral cancer is usually defined as neoplasm of lip, tongue and intra oral tissue including the oropharynx (Gurlanick1984). Although the disease has been reported in various age group but it occurs frequently in patients over the age of 60 years (Najjar and Gatson 1980). In India and Sri Lanka the oral cancer cases almost 30 – 50 percent of all cancers in males whereas in most western countries the disease accounted for only 2- 6 percent of all cases of cancer (Pinborg 1977). The morbidity and mortality rates of the disease vary with various countries, culture within countries, geographic areas, occupation and ethnic background (Pindborg 1980). There are many risk factors related to the oral cancer, namely tobacco, alcohol, nutritional deficiencies, chronic candidosis, betel quid chewing and

chronic oral irritation. Tobacco is a traditionally high risk factor for oral cancer (Jayant et al.1977; Rothman 1978; Mehta et al. 1981). Both volatile and non-volatile carcinogens, N – nitrosamines, have been detected in tobacco and tobacco smoke (Brunnemann et al. 1977 and Hoffman et al. 1979). The mechanism(s) by which alcohol promotes carcinogenesis is not clear, but it may involve the promotion of nutritional deficiencies or some co carcinogens in alcoholic beverages. The close relationship between siderophenic dysphagia (Patterson – Kelly or Plummer –Vinson syndrome) caused by chronic iron deficiency and oral cancer has been demonstrated (Wynder et al . 1957; Watts 1961; Larrson et al. 1975). The high incidence of oral cancer in Central and South East Asia has for long been linked with the habit of betel nut chewing particularly when tobacco has been incorporated into the quid. It has shown that in India and Sri Lanka there was a low incidence (2.02%) of oral cancers in betel nut chewers whereas an elevated incidence of disease (49.9%) was demonstrated in patients who had habitually chewed both betel nut and tobacco (Hirayama1966). The difference between cancer and non cancer patients addicted to smoking and drinking was not significant but the difference in betel quid chewing was great. There is no definite evidence that betel nut has a direct carcinogenic action, it is believed that chronic irritation and continuous friction of the cheek against the betel quid and and the sharp edges of the abraded teeth may cause traumatic ulcer, leukoplakia and even malignant transformation. The changed that occur in the oral mucosa as a result of betel quid chewing in individuals who have or have not yet developed carcinomas and the incidence of such changes have been studied in different parts of the world where the habit is widespread (Pindborg et al.1968; Lee and Chin 1970; Reichart et al. 1984).studies in which small number of betel quid chewers and non chewers were compared have shown an increased occurrence of micronuclei in exfoliated cell in oral mucosa and sister chromatid exchanges in peripheral lymphocytes from chewers(Stich and Rosin 1984; Ghosh and Ghosh 1984). Compounds that have been detected or may be present in the saliva of betel quid chewers include arecoline (Panigrahi and Rao1982), arecaidine (Panigrahi and Rao 1984), eugenol (Stich et al. 1981) and anatabine (Riebe and Westphal 1983), all of which have shown to exert genotoxic effects. A longer duration of betel chewing may increase a chance of neoplastic development in the oral cavity. Some betel nut specific N-nitroso compounds such as N- nitrosoguavacline reported in the saliva of betel quid chewers.Nicotene, cotinine and tobacco specific nitrosamines were also detected in the saliva of chewers of betel nut with tobacco and in that of chewers of tobacco (Wenke et al.1984; Nair et al. 1985). The result suggests that N – nitrosoguavcoline (NG), 3 – (methyl nitrosamino) propionitrile (MNPN) and 3 – (methyl nitrosamino) propionaldehyde (MNPA) could be formed by N- nitrosation of arecoline, the major alkaloid in betel nut. Formation of NG occurred probably by oxidative cleavage of N – methyl bond, similar to the reaction which other cyclic tertiary amines such as N –methyl piperdine, N –methylpyrrolidine or nicotine underwent with nitrite. In case of MNPN, it was suggested that the cyano group of MNPN did not stem from the thiocyanate ion because MNPN could be formed in the absence of the ion. Shivapurkar et al. (1980) indicated that users of betel nut without tobacco showed an increase of salivary nitrite during chewing in contrast to those who chewed betel nut product with tobacco who reflected even lower nitrite levels in saliva than non chewers. It was also indicated that in a few cases that salivary thiocyanate remained rather constant (0.2 – 0.5M) during chewing while the pH changed from slightly acidic to neutral. These condition will facilitate the formation of nirtosamines from arecoline during betel quid chewing.

CONCLUSION

The findings suggest that in betel quid chewing condition, arecoline must react with the methyl ester group of cystiene and be converted to arecaidine by hydrolysis with lime. The reaction of arecoline and arecaidine with thiols group indicated that they were biological alkylating agent. Such a property was a feature of many chemical reactions either with or without metabolic activation (Lawley 1980). Feeding Swiss mice with above constituents showed that betel nut and betel quid produced lung tumors in 47 % and 26% of treated animals. In fact there have been several reports which demonstrated carcinogenicity of betel quid in experi-

mental animals . Mitotic index is most prognosis factor of oral cancer. We have screened 209 cases, out of which 60.28% had betel quid chewing habit.

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