



## EFFECT OF TEA ON PERCENTAGE OF MICRONUCLEI OF CASES WITH ORAL LESION FROM EASTERN INDIA

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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 (*Areca catechu*), a major component of BQ, contains certain alkaloids that give rise to nitrosamines. Mitotic index (MI) and Micronuclei (MN) were studied among the studied population. In this present study subjects were screened from Department of E.N.T. & Oral and Maxillofacial surgery of RKMS hospital, Kolkata and different areas of Eastern and North Eastern states of India. Exfoliated cell from the buccal mucosa were examined for micronuclei (MN). Some of them had more than one micronucleus. It has been found that micronuclei percentage was higher. Most of the subjects had betel quid chewing habit. Micronuclei percentage was low after supplementation of tea. Betel quid has a vital role in developing oral cancer. Tea has a chemo preventive property for polyphenol content.

**KEY WORDS:** Oral cancer, betel quid, tea, micronuclei *etc.*

### INTRODUCTION

Tea has received a great deal of attention because tea polyphenols are strong antioxidants, and tea preparations have shown inhibitory activity against tumour induction. Tea polyphenols, known as catechins, usually account for 30–42% of the dry weight of the solids in brewed green tea. Antioxidant properties of tea have been partially resulted in several health benefits. (Wiseman *et al.*, 1997; Rice Evans, 1999). The major polyphenolic components of black tea (the fermented product) are theaflavins (1–3% dry weight) and thearubigins (10–40% dry weight). A study suggests that black-tea derived polyphenols also showed one of the chemo-preventive properties shown by green tea polyphenols. Black tea polyphenol fractions hamper the formation of [3H]-B (a) P-derived DNA adducts as well as the activity cytochrome P450 isozymes (Krishnan & Maru, 2005). Oral squamous cell carcinoma (OSCC) is the eighth most common cancer worldwide and well defined subgroup of head and neck cancer (Boyle & Ferlay, 2004; Ferlay *et al.*, 2007). All parts of the oral cavity are susceptible to cancer from tobacco smoking or chewing, including lip, tongue, palate, gum and cheek (Taybos, 2003; Silverman, 2003). The main carcinogens in pan masala and gutkha are derived from their ingredients; areca nut, catechu, lime and tobacco. Areca nut (*Areca catechu*), a major component of BQ, contains certain alkaloids that give rise to nitrosamines, some of which such as *N*-nitrosoguvacoline, 3-(methylnitrosamino) propionitrile, 3-methylnitrosamino propionaldehyde and *N*-nitrosoguvacine, are shown to be carcinogenic (Hoffmann *et al.*, 1994). Several workers have investigated the chemo preventive action of tea against cancer. (Lambert & Yang, 2003; Talukder & Sharma, 2004; Mukherjee *et al.*, 1999). Reactive oxygen species (ROS) implicated in multistage carcinogenesis, are generated in substantial amounts in the oral cavity during

chewing (Nair *et al.*, 1992). ROS concentration will increase in the oral cavity of chewers as soon as the areca nut and catechu polyphenols together with slaked lime dissolve in the saliva, similar to the reaction observed in vitro (Nair *et al.*, 1987). There is an increasing effort world-wide to determine the impact of environmental, genetic and life-style factors on genomic stability in human populations. As a result of rapid globalization and changing social attitudes, tobacco and betel quid chewing habits have been increasing worldwide. Tobacco chewing along with various ingredients like areca nut, catechu, lime, cardamom, permitted spices, unspecified flavouring agents have been reported to possess cytotoxic, mutagenic and genotoxic properties (IARC, 1985; Panigrahi & Rao, 1986; Wary & Sharan, 1988). The formation of micronuclei in the eukaryote cells is an end point of chromosomal damage or segregation errors (Geard & Chen, 1990), the presence of micronuclei reflects a genotoxic or carcinogenic exposure. Due to its association with chromosomal aberrations, micronuclei have been used since 1937 as an indicator of genotoxic exposure, based on the radiation studies conducted by Brenneke and Mather (Heddle *et al.*, 1983). The assay is reliable and technically easy to perform. The direct correlation between the micronuclei formation and genomic damage make the micronuclei assay an efficient alteration to the metaphase analysis (Fenech *et al.*, 1990). The present study was carried out by the Department of Genetics in collaboration with the Departments of ENT and Faciomaxillary surgery, of the Ramakrishna Mission Seva Pratishthan Hospital, Kolkata.

### MATERIALS AND METHODS

#### Screening of Subjects

Screening of subjects was carried out in 3 settings:

Camp in Eastern India, Camp in North East India and subjects attending Maxillofacial and ENT department of RKMSH hospital.

An informed consent were taken from all the subjects and approval by the Institute's Ethics Committee

I) Eastern India camp: - 220 subjects were screened at a camp held in Bankura, Purba Midnapur, Atghara in West Bengal. Out of whom, 133 were betel quid chewers.

II) North East camp: - 56 subjects were screened at a camp held in Karimganj, Assam. Out of whom 33 were betel quid chewers

III) RKMSH Hospital: - 2606 subjects attending in one year at E.N.T OPD and Oral Maxillofacial OPD of RKMSH Hospital had other complications like auditory, nasal, throat & facial problem. 35 subjects were selected for our study. All subjects were administered a

standardized questionnaire interview to obtain any history of relevant risk factors and addictions.

**Oral smears were obtained from the subjects as follows**

The subjects were asked to rinse their mouths with water and a pre moistened wooden spatula was used to sample cells from the oral mucosa. The spatula was applied to a pre cleaned microscope slide. Smears were air dried and fixed in 80% methanol. Slides were stained by the Giemsa solution and the MN frequency was scored (Sarto *et al.*, 1990; Tolbert *et al.*, 1992). The same person scored 1000 cells blindly in each case to determine the MN percentage.

**RESULTS**

Total 311 subjects studied (Table 1), out of which 56 subjects from Karimganj, Assam, 220 subjects from Eastern India and 35 subjects from RKMSH hospital.

**TABLE 1: Detailed history of subjects of different areas**

PLACE	NO	AGE GROUP ( in years)					Addiction						
		Below 30	31-40	41-50	51-60	61-70	Above 70	Smoking	Alcohol	Betel Quid	No BQ Addiction	Tea Drinker	Non Tea Drinker
NORTH EAST CAMP	56	1	2	12	24	11	6	9	6	33	23	40	16
1. Assam, Karimganj													
EASTERN INDIA CAMP													
1) Bankura,Dhulai	34	5	20	8	1	0	0	16	14	19	15	34	0
2)East Midnapur, Bibhisanpur	46	22	13	3	6	2	0	28	29	36	10	40	6
3) North 24 Pgs, Atghara	89	28	18	21	15	6	1	27	3	56	33	73	16
4)Narrah, Bankura	51	8	13	12	8	6	4	14	5	22	29	49	2
RKMSH	35	2	7	8	11	7	0	20	8	24	11	29	6
TOTAL	311	66	73	64	65	32	11	114	65	190	121	265	46

Some of them had more than one addiction.

Out of 56 subjects from North East 58.92% were betel quid chewers and 71.42% were tea drinker. They took betel quid more than once. Out of 33 betel quid chewers, 6 cases took betel quid occasionally, rest of them took BQ 3-4 times /day. Out of 34 subjects from Dhulai, Bankura, 55.88% were betel quid chewers and all of them were tea drinker. Out of 19 betel quid chewers, 13 cases took betel quid occasionally, rest of them took BQ 2-5 times / day. Out of 46 subjects from East Midnapore, 78.26% were betel quid chewers and 86.95% were tea drinker. Out of 36 betel quid chewers, 5 cases took betel quid occasionally, rest of them took BQ 7-16 times /day. Out of 89 subjects from North 24 Parganas, 62.92% were betel quid chewers and 82.02% were tea drinker. Out of 56 betel quid chewers, 19 cases took betel quid occasionally; rest of them took BQ 6 -10 times /day. Out of 35 subjects from RKMSH hospitals 68.57% were betel quid chewers and 82.85% were tea drinker. Out of 24 betel quid chewers, 12

subjects took betel quid occasionally, rest of them took BQ 4-9 times /day. From (Table 2) micronuclei value was higher at Dhulai, Bankura than other area because of their life style; they took betel quid as mood elevator and also as habit.Percentage of Micronuclei before supplementation of Tea were  $13.86 \pm 2.70$  and after supplementation of Tea were  $3.05 \pm 0.59$ . (Table 3)

**TABLE 2: Micronuclei of different studied population**

PLACE	Micronuclei (Mean ± SE)
Dhulai, Bankura	26.29 ± 1.95
Bibisanpur, East Midnapore	12. 31 ± 2.75
Atghara,North 24 Pgs	4.76 ± 1.26
RKMSH Hospital	*****
Narrah, Bankura	4.61 ± 2.82

\*\*\*\*\* Study of micronuclei of oral cancer cases was not possible due to severe ulceration and bleeding and cases were unable to open their mouth

**TABLE 3: Percentage of Micronuclei before and after supplementation of Tea**

Before supplementation of Tea (Mean ± SE)	After supplementation of Tea (Mean ± SE)
13.86 ± 2.70	3.05 ± 0.59

### Black tea supplementation

The subjects were advised to drink 3 cups of tea per day brewed with approximately one teaspoonful of tea in 100 ml of water. Subjects were asked not to add milk in it or not to boil it for long time. It was advised to keep tea liquor inside the oral cavity for 1 – 2 mins and then drink it. The polyphenols content of the supplied tea is  $28 \pm 1.86$  / 100 gm of dry tea. Tea was supplied by National Tea Research Foundation (NTRF) funded agency, Kolkata, India.

### DISCUSSION

Oral cancer is one of the leading cancers in most Asian countries (Lin *et al.*, 2002). In another study the prevalence of head and neck cancer was found to be significantly high at 54.48% in the population of North Eastern India. The use of a biomarker as an indicator of disease development is that the marker will translate into a relationship between exposure and disease (Schatzkin *et al.*, 1990). Micronucleus (MN) has been used since 1937 as an indicator of genotoxic exposition based on radiation studies conducted by Brenneke and Mather, as reported by Heddle *et al.* (1983). Since the prolonged use of the chewing items such as supari, pan masala / gutkha can generate a risk of developing different types of oral cancer, it becomes necessary to screen the population for its possible risk. Casartelli *et al.* (2000) observed MN frequencies in exfoliated buccal cells in normal mucosa, precancerous lesions and squamous cell carcinoma. They concluded that the gradual increase in MN counts from normal mucosal to precancerous lesions to carcinoma suggested a link of this biomarker with neoplastic progression. In our study we have seen that percentage of micronuclei was  $13.86 \pm 2.70$ . After supplementation of tea for 6 months we have seen that percentage of micronuclei was  $3.05 \pm 0.59$  and the area of white patches or leukoplakia was reduced in size. The antioxidant properties of tea play a vital role to reduce the cancer biomarker. The polyphenolic component of tea decreases its antioxidant property with combination of milk. The milk protein casein binds the antioxidant portion of the tea and reduces its property. So black tea i.e. tea without milk plays an important role for scavenging the free radicals and promote good health.

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