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Research article

## STUDY AMONG BETEL QUID CHEWERS FROM INDIAN POPULATION

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### ABSTRACT

**Background:** Oral cancer is most common in males and also in females. Betel quid (BQ) is the main causative agent of oral cancer. 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. **Methods:** In this present study subjects were screened from Department of E.N.T. & Department of Oral and Facio maxillary of RKMS hospital, and different areas of Eastern and North Eastern states of India. For mitotic index (MI) blood leukocyte cultures were analyzed and for micronuclei (MN) buccal mucosa were examined. **Results:** Some of them had more than one addiction. Micronuclei percentage and mitotic index both higher than normal. **Conclusion:** Betel quid play a role in changing the oral pathology and thus causes oral cancer.

**Keywords:** Oral cancer, betel quid, micronuclei, mitotic index

### INTRODUCTION

Oral premalignancies are very common in betel quid chewers and is the most common cancer worldwide. 45% and 60% mortality depend upon the patient group and the disease frequently associated with tobacco smoking, chewing. Lip, tongue, palate, gum and cheek these parts of the oral cavity are effected from tobacco chewing . Malignancies of the oral cavity arise from the precancerous lesion such as leukoplakia, erythroplakia and pre cancerous condition such as oral submucous fibrosis. Oral squamous cell carcinoma and the most common oral premalignancies such as leukoplakia and oral submucous fibrosis appear to be related to the habit of betel quid (BQ) chewing in South East

Asia, whereas in Western countries cigarette smoking and heavy alcohol consumption are the main causative agents. Areca nut (Areca catechu), a major component of BQ. Areca nut 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.<sup>1</sup> These BQ-specific nitrosamines may act as an adjunct to tobacco-specific nitrosamines that are strongly implicated as an etiologic factor for leukoplakia and oral submucous fibrosis. Metabolic activation of several nitrosamines was reported to be catalyzed

by cytochrome P450 enzymes (CYPs), large multi-gene family enzymes important in phase I metabolic activation reactions. Furthermore, reactive oxygen species are generated in the oral cavity during BQ chewing due to the addition of slaked lime  $[Ca(OH)_2]$  into BQ.<sup>2</sup> Among xenobiotic metabolizing enzymes, the CYP2A family is characteristic of its catalytic properties to nitrosamines.<sup>3</sup> Several workers have investigated the chemopreventive action of tea against cancer.<sup>4,5,6</sup> Consumption of black tea has been shown to exert a protective effect against oral precancerous lesions.<sup>7</sup> Epigallocatechin-3-gallate (ECG), is one of the major component of green tea which inhibit cell growth and also reduce tumor incidence. Since the formation of micronuclei in the eukaryote cells is an end point of chromosomal damage or segregation errors.<sup>8</sup> The presence of micronuclei reflects a genotoxic or carcinogenic exposure. Association with chromosomal aberrations, micronuclei acts as a good indicator of genotoxic exposure. The assay is reliable and technically easy to perform. The present study was carried out by the Department of Genetic Toxicology, in collaboration with the Departments of ENT and Oral Maxillofacial surgery, of the Ramakrishna Mission Seva Pratishthan (RKMSp) Hospital, Kolkata. The purpose of this study is to see whether percentage and chromosomal damage are more in oral cancer.

## PATIENTS AND METHODS

After the Institutional Ethics committee approval, study was conducted in ENT and Oral Maxillofacial department of RKMSp Hospital.

**Inclusion criteria:** Screening of subjects was carried out in 3 settings:-

I) Camp in Eastern India: 220 subjects were screened at a camp held in Bankura, Purba Midnapur, North West Bengal. Out of them, 133 were betel quid chewers were included in the study

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

III) RKMSp Hospital: 35 subjects were screened out of them 24 cases were betel quid chewers.

Out of 24, 7 subjects had pre cancerous lesion, 6 subjects had squamous cell carcinoma, 11 subjects had pre cancerous condition.

Total 311 subjects were screened from different areas of Eastern India, North East India and E.N.T and Oral Maxillofacial OPD of RKMSp hospital. Among them total 190 subjects included for study who were betel quid chewers, from each person conform consent was taken.

**Exclusion criteria:** The subjects who had no betel quid chewing habit. The subjects who had betel quid chewing habit and any type of precancerous lesion, precancerous condition related with betel quid was included in this study. Screening of subjects was carried out by administering questionnaires.

**Methods:** Detailed history was taken from all cases by filling up questionnaire.

**Parameters studied:** Leukocyte culture, Micronuclei (MN) study

**Leukocyte culture:** - Peripheral blood was collected from all subjects. Human leucocyte culture was carried out by the method of Moorhead PS et al<sup>9</sup> modified by the method of Sharma and Talukder.<sup>10</sup> A total of 4 ml of peripheral venous blood was collected from each patient under aseptic condition with the help of a sterile disposable needle and transferred to a heparinized vial. Leucocyte rich plasma (0.5 ml) was added to a 5 ml of culture media (RPMI 1640, Sigma, St. Louis, USA) supplemented with 20 % foetal bovine serum (Sigma) and Phytohaemagglutinin M (0.04 ml / ml of culture media, GIBCO BRL). The cultures were incubated at 37° C. After 70 hours incubation, colchicines (0.2 ml of 0.04% / ml) was added. Two hours later, cells were centrifuged at 1000 rpm for 5 min, treated with prewarmed KCL (0.075M) for 15 min, centrifuged at 1000 rpm for 5 min, and fixed in methanol: acetic acid (3:1).

Fixed cell suspension was taken on clean grease free glass slide and air dried. The preparation was stained with aqueous Giemsa. All slides were coded and 1000 blast cells were scored to determine mitotic index per individual.

Micronuclei (MN) study: - For Micronuclei study Premoistened wooden spatula was used to sample cells from the oral mucosa. The spatula

was applied to a precleaned microscope slide. After air dried the smear fixation was done by methanol. Slides were stained by the Giemsa solution and the MN frequency was scored using the criteria described by Sarto et al.<sup>11</sup> The same person scored 1000 cells blindly in each case to determine the MN Percentage.

## RESULTS

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

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

**Table 2: Micronuclei and Mitotic Index of betel quill chewers.**

PLACE	Micronuclei (%) (Mean ± SE)	Mitotic Index (Mean ± SE)
Dhulai, Bankura	26.29 ± 1.95	3.94 ± 0.23
Bibisanpur, East Midnapore	12.31 ± 2.75	8.66 ± 0.67
Atghara	4.76 ± 1.26	5.07 ± 0.60
RKMSP Hospital	----- *	4.54 ± 0.33
Narrah, Bankura	4.61 ± 2.82	4.28 ± 0.62

(Normal values of MI are < 4% and MN is < 1%)

\*Study of micronuclei of oral cancer cases was not possible due to severe ulceration and bleeding and cases were unable to open their mouth. Mean value of mitotic index of all cases having betel quid chewing habit was 5.29 ± 0.49 and mean percentage of micronuclei were 12 ± 2.19

Total 311 subjects studied (Table 1), out of which 56 subjects from North East camp (Karimganj, Assam), 220 subjects from East India and 35 subjects from RKMSp hospital. 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 Pgs 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 RKMSp 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.

90% subjects were coming from rural areas at Karimganj. All subjects were from Dhulai, Bibhisapur, Narrah, Atghara under rural areas. 10% subjects were coming from rural areas of RKMSp Hospital and rests on them were from urban areas.

Mean value of mitotic index (MI) of all cases having betel quid chewing habit was  $5.29 \pm 0.49$  and mean percentage of micronuclei (MN) were  $12 \pm 2.19$  (Table 2)

## DISCUSSION

According to World Health Organisation (WHO) data, the standardized mortality rate for 2002 was 2.2 deaths per 100,000 populations. Oral cancer is one of the leading cancers in most Asian

countries.<sup>12</sup> In another study the prevalence of head and neck cancers was found to be significantly high at 54.48% in the population of North Eastern India. Gurkha and pan masala has been shown to be carcinogenic in experimental animals, causing tumors in various organs. No effective techniques have yet been developed for making direct chromosome preparation from epithelial tissues. They concluded that the gradual increase in Clinical chemoprevention trials on oral pre-malignancies have used MN in oral mucosa as a surrogate endpoint of cancer.<sup>13</sup> These findings clearly suggest a causal link between MN and cancer. Micronuclei are the small extra nuclei cells which is formed in metaphase and anaphase stage. The presence of micronuclei reflects a genotoxic and carcinogenic exposure since it is associated with chromosome aberrations.<sup>14</sup> Micronuclei have been used as an important marker. In our study we have seen that mitotic index of all subjects having betel quid chewing habit and mean percentage of micronuclei were higher than normal. Normal values of MI are  $< 4\%$  and MN is  $< 1\%$ .

M. Sulkowska,<sup>15</sup> observed that mitotic index count was high in oral squamous cell carcinoma cases. Mitotic activity has proven to be an efficient prognostic indicator of squamous cell carcinoma of various sites.

## CONCLUSION

Betel quid has an immense role in changing the oral pathology and developing oral cancer. In this present study it has been found that the micronuclei percentage can be used as a biomarker. The Micronuclei percentage and mitotic were higher than normal.

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