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Research Article

CHROMOSOMAL STUDY AMONG BETEL QUID CHEWERS OF INDIAN POPULATION

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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 nuts, Catechu, 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. 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. Mitotic index are higher in cancer and pre cancer (both betel quid and non betel quid chewers) cases than normal. Percentage of chromosomal aberration (CA) is higher in cancer cases.

INTRODUCTION

The betel plant is an evergreen perennial, with glossy heart-shaped leaves, originated in South and South East Asia. The primary use of betel leaf is as a wrapper for the chewing of areca nut or tobacco where it is mainly used to add flavour. In the Indian states of Karnataka, Kerala, Tamil Nadu, Andhra Pradesh, Maharashtra, Assam, West Bengal and Odisha a sheaf of betel leaves is traditionally offered as a mark of respect and auspicious beginnings. Occasions include, greeting elders at wedding ceremonies, New Year, offering payment to Ayurvedic physicians and astrologers where usually money and/or areca nut are kept on top of the sheaf of leaves and offered to the elders for their blessings. The term 'betel quid' is often used with insufficient attention given to its varied contents and practices in different parts of the world. A 'betel quid' (synonymous with '*pan*' or '*paan*') generally contains betel leaf, areca nut and slaked lime, and may contain tobacco. Other substances, particularly spices, including cardamom, saffron, cloves, aniseed, turmeric, mustard or sweeteners, are added according to local preferences. According to the CDC (Centers for Disease Control and Prevention), betel plant, areca nut, and betel quid usage causes an increased risk of developing white or reddened lesions in the mouth that can progress to cancer. Use is also responsible for a condition called oral submucous fibrosis (OSF), in which inflammation and scarring (fibrosis) develop in the tissues lining the mouth, but can progress to affect the esophagus (swallowing tube). This is a debilitating and irreversible condition that, as it progresses, results in a stiff jaw and an inability to open the mouth.

Oral cancer is the third most common cancer in India. Nearly, 1,30,000 Indians die due to tobacco related oral cancer. Oral cancer is mainly attributed to the use of chewing betel quid and tobacco since, Indians chew tobacco than smoke it, due to which 75,000 to 80,000 new oral cancer cases have been identified in 2012 and these proportions will increase further by 2025

(Ferlay *et al*; 2013).Oral cancers account for the highest cancer related mortality among men aged 30-69 in India (Dikshit *et al*; 2012). Chewing of the betel quid (often referred to as "paan") is fairly common; the quid is usually made up of areca nut, catechu, slaked lime, and often tobacco, which are placed in a betel leaf and folded into the characteristic triangular shape. Chewing paan releases carcinogenic nitrosamines from the arecanut that can cause pre- neoplastic changes (IARC 2012).

The Indian Council of Medical Research (ICMR) said in 2016 the total number of new cancer cases is expected to be around 14.5 lakh and the figure is likely to reach nearly 17.3 lakh new cases in 2020. Over 7.36 lakh people are expected to succumb to the disease in 2016 while the figure is estimated to shoot up

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to 8.8 lakh by 2020. Data also revealed that only 12.5 per cent of patients come for treatment in early stages of the disease.

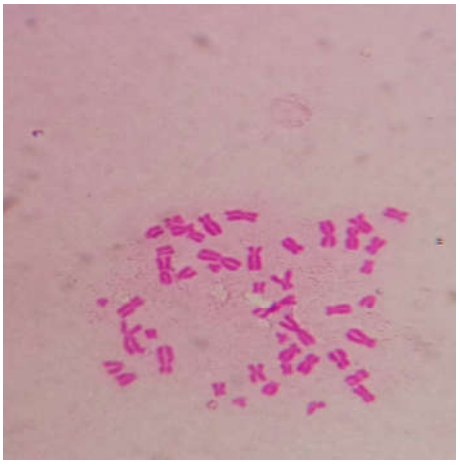
MATERIALS AND METHODS

A case control study was conducted on all cases with cancer and pre cancerous who were referred to the different areas of West Bengal, Kolkata. 30 age sex matched control cases were recruited as healthy human being from camp of West Bengal.

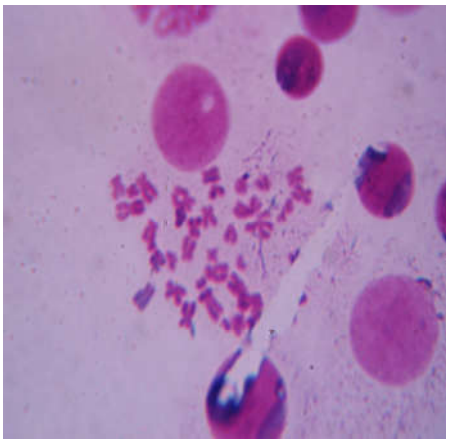
1. Detailed history was taken from all cases by filling up questionnaire.
2. Leukocyte culture:-

Human leukocyte culture was performed followed as the method modified from Moorhead *et al*. 1960 (Moorhead *et al*; 1960, Sharma and Talukder 1974).5 ml peripheral blood samples were taken from each subject in heparinised vials by vein puncture. The blood samples were coded for lymphocyte culture. Leukocyte culture was carried out for chromosomal aberration (CA) by the method of Sharma and Talukder 1974. For each subjects duplicated culture were maintained. Leukocyte rich plasma were added to 5ml culture media supplemented with 20% fetal bovine serum and Phytohaemagglutinin M (0.04ml/ml of culture media). The culture were incubated at 37°C. The harvesting was done at 72hrs after initiation of culture. At 70 hrs of culture, colchicine was added. Two hrs later cells were centrifuged at 10000 rpm for 10 min and fixed in methanol and glacial acetic acid (3:1). Fixatives were removed by centrifugation and two more changes of fixative were performed. Fixed cell suspension was laid on the 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. All slides were coded and 100 metaphase plates were scored randomly for chromosomal aberration.

Inference: In our study mitotic index are higher in cancer and pre cancer (both betel quid and non betel quid chewers) cases than normal.



Normal human chromosome



Chromosomal abberrations

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			
North East Camp 1. Assam, Karimganj	56	1	2	12	24	11	6	9	6	33	23	40	16
Eastern India Camp	34	5	20	8	1	0	0	16	14	19	15	34	0
1. Bankura, Dhulai	46	22	13	3	6	2	0	28	29	36	10	40	6
2. East Midnapur, Bibhisanpur	89	28	18	21	15	6	1	27	3	56	33	73	16
3. North 24 Pgs, Atghara 4. Narrah, Bankura	51	8	13	12	8	6	4	14	5	22	29	49	2
RKMSP	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

Note: Some cases had more than one addiction

Table 2 Percentage of mitotic index of studied cases and healthy control

	Healthy Control	Cancerous		Pre cancerous	
		With betel quid	Without betel quid	With betel quid	Without betel quid
Mitotic index	1.31±0.15	3.31 ± 0.41	2.64 ± 1.61	3.03 ± 0.4	2.02 ± 0.74

Table 3 Chromosomal aberration (CA) of studied case and healthy control

Cancer with betel quid (Mean ± S.E.)	Cancer without betel quid (Mean ± S.E.)	Pre Cancer with betel quid (Mean ± S.E.)	Pre Cancer without betel quid (Mean ± S.E.)	Healthy Control (Mean ± S.E.)
0.58 ± 0.06	0.41 ± 0.26	0.44 ± 0.08	0.32 ± 0.21	No Aberration

Inference: Percentage of chromosomal aberration (CA) are higher in cancer cases who had betel quid chewing habit and are less in pre cancer cases without betel quid chewing habit. There is no chromosomal aberration (CA) was seen among healthy control.

DISCUSSION

M. Sulkowska, observed that mitotic index count was high in oral squamous cell carcinoma cases. (Sulkowska *et al*; 2003) mitotic index is simply a measurement to determine the percentage of cells undergoing mitosis Mitotic activity has proven to be an efficient prognostic indicator of squamous cell carcinoma of various sites. Mitotic index are higher in cancer and pre cancer (both betel quid and non betel quid chewers) cases than normal. Percentage of chromosomal aberration (CA) is higher in cancer cases. There is no chromosomal aberration (CA) was seen among healthy control.

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 elevated mitotic index indicates more cells are dividing, and thus obvious in cancer cells.

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