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STUDY OF CYP2A6 GENE POLYMORPHISM AMONG BETEL QUID CHEWERS OF INDIAN POPULATION.

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ABSTRACT
Introduction: Oral cancer is most common cancer in males and third most common in females, one of 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. CYP2A6 genetic polymorphism were studied among the Eastern and North eastern indian population. **Methods:** In this present study subjects were screened from Department of E.N.T. & Oral and Maxillofacial surgery of RKMSP hospital, Kolkata and different areas of Eastern and North Eastern states of India. Polymorphism of CYP2A6 gene was studied from EDTA blood. **Results:** Some of the cases had more than one addiction. It has been found that most of the subjects had betel quid chewing habit. Early metabolizer are susceptible to oral cancer where as in case of poor metabolizers chances are less. **Conclusion:** Betel quid has a role in changing the oral pathology.

KEYWORDS: Oral cancer, Betel quid, CYP2A6 polymorphism.

INTRODUCTION
Oral cancer refers to a subgroup of head and neck malignancies that develops in lips, tongue, salivary glands, oropharynx, buccal surface and other intra oral lesion. The betel quid chewing habit has been reported from many countries including India. Betel quid is a combination of betel leaf, areca nut, slaked lime (aqueous calcium hydroxide paste). There are several forms of areca nut (green unripe, ripe but raw, baked roasted or boiled). Chewing of areca nut with betel quid was the major etiologic factor for oral leukoplakia (OL) and oral submucous fibrosis (OSF).[1] CYPs (Cytochrome P450s) are important in detoxification and metabolic activation of numerous foreign chemicals. Polymorphism of CYP enzymes may changes the enzymatic activites. CYP2A6 catalyzes the oxidation of nicotine and activation of carcinogens and nitrosamines. Based on the genetic variation subjects are classified as EM (Early metabolizer) and PM (Poor metabolizer).

MATERIALS AND METHODS
1. Materials
Total 3995 cases were screened from Eastern and North Eastern India. Out of these cases 3105 were from Eastern India and 890 cases were from North Eastern states. 266 cases had betel quid chewing habit. Out of 266 cases 157 were from eastern India and 109 cases were from North Eastern states such as Karimganj, Silchar, Assam and Shillong, Meghalaya.

132 cases had oral lesion among North East population of whom 103 cases had betel quid chewing habit. 255 cases had oral lesion among East India population out of which 157 had betel quid chewing habit.

An informed consent were taken from all the subjects.

II. Methods
i) Detailed history was taken from all cases by filling up questionnaire.
ii) Isolation of blood 3 ml peripheral blood was taken from each cases and DNA was isolated by DNA isolation mini kit. (QIAGEN, Germany).
iii) Molecular study PCR was performed with forward and reverse primer at 58°C for annealing temperature with 35 cycles. Total amount of PCR product is 26.5µL.

The project code is 2438/2012. The date of the permission of the ethical committee: 2nd August, 2011 and name of the ethics committee is The ethical committee of the Ramakrishna Mission Seva Pratishthan, Vivekananda Institute of Medical Sciences, Kolkata.

RESULT

Table 1a: Detailed history of subjects from North East India.

PLACE	NO of Cases who had oral Lesion	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
2. Silchar, Assam	37	5	10	13	4	3	2	12	12	34	3	32	5
3. Shillong, Meghalaya	39	14	8	9	4	2	2	9	28	36	3	37	2
TOTAL	132	20	20	34	32	16	10	30	46	103	29	109	23

Table 1b: Detailed history of subjects from Eastern India.

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			
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	255	65	71	52	41	21	5	105	59	157	98	225	30

Total 387 cases who had oral lesion was included in this study. Out of which 132 cases from North East and 255 cases were from Eastern Indian states.

103 cases out of 132 cases had betel quid chewing habit among North East population i.e. 78.03% and 157 cases had betel quid chewing habit among Eastern Indian population i.e. 61.5% (Table 1a and 1b).

Table 2: Poor metabolizer and Early metabolizer of different area.

Area	No of betel quid chewers	Poor metabolizer	Early metabolizer
Dhulai, Bankura	19	16%	84%
Bibisanpur, East Midnapore	36	42%	58%
Atghara, North 24 Pgs	56	90%	10%
RKMSP Hospital	22	13%	87%
Narrah, Bankura	24	18%	82%
Silchar, Assam	33	17%	83%
Shillong, Meghalaya	19	33%	67%
Karimganj, Assam	33	60%	40%

Table 3: Percentage of CYP2A6 Polymorphism among studied population.

AREA	CYP2A6 Gene Polymorphism	Normal CYP2A6 Gene
North east camp	63.3%	36.7%
Eastern camp	35.8%	64.2%

CYP2A6 gene polymorphism was present among 63.3% among North East population and 35.8% polymorphism was present among Eastern Indian Population. Early metabolizer i.e. normal CYP2A6 are susceptible to oral cancer whereas in case of poor metabolizer chances are low.

DISCUSSION

The incidence of oral cancer has significant local variation and is increasing in some parts of the world. In India and other Asian countries, Oral and oropharyngeal carcinomas comprise up to half of all malignancies and this high prevalence is due to the influence of carcinogen and region specific epidemiological factors, especially tobacco and betel quid chewing.

In Western countries consumption of tobacco[2] and alcohol[3] is the risk factors for development of oral cancer whereas in Asian countries the use of smokeless tobacco products such as gutkha, panmasala and betel quid[4,5] is responsible for a considerable percentage of oral cancer cases.

It is estimated that at least 200 million individuals consume areca nuts in one form or another worldwide. The habit is now widespread in Southeast Asia and the South Pacific islands and in people of Indian origin elsewhere in the world. The betel quid chewing habit is in fact found all over the world wherever Indians have settled. 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.[6] The major areca nut alkaloids are arecoline, arecaidine, arecolidine, guvacoline and guavacine.[7] 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.[8] 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 nonhomogeneous. 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.[9] Oral sub mucous fibrosis (OSMF) is a chronic condition characterized by mucosal rigidity of varying intensity due to fibro elastic transformation of the juxta epithelial layer.[10] Areca nut chewing could be one of the most important etiologic factors in OSMF.[11] The areca nut, the major constituent of pan masala is responsible for mutagenic, clastogenic and carcinogenic properties.[12] Chewing of tobacco with BQ results in high exposure to carcinogenic tobacco-specific nitrosamines (TSNAs), to 1000 mg/day[13], compared with 20 mg/day in smokers[14], as well as leading to exposure to nitrosamines derived from areca nut alkaloids.

There are estimated 600 million BQ users globally. The Cytochrome p450 (*CYP*) families are divided into14 gene families of which CYP1, CYP2 and CYP3 are primarily active in the metabolism of a wide range of chemicals and these CYPs families are implicated in the metabolic activation of BQ and areca nut-specific nitrosamines.[15] CYPs are located on human chromosome19. The CYP2A6 gene consist of 350 kilobases located at 19q 12 – 19q 13.2.[16-18] The CYPs that are known to exhibit polymorphism are CYP1A1, CYP2A6, CYP2C9 and CYP2E1.Out of this polymorphism of CYP1A1, CYP2A6 and CYP2E1gene have been studied in relation to susceptibility to head and neck cancers. Genetic defects in the CYP2A6 gene may also effect susceptibility to pre carcinogen in the environment. People are classified as EM known as early metabolizers and PM known as poor metabolizers based on genetic variation.[19] The PM phenotype are incapable of metabolizing the exogenous compound but EM phenotype are capable of metabolizing the exogenous compound.[20,21]

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