RELATION WITH ORAL SQUAMOUS CELL CARCINOMA AND BETEL QUID: A MOLECULAR CYTOGENETICS STUDY.

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Introduction:
Betel quid (BQ) chewing is widely prevalent in India and many parts of Asia. The International Agency for Research on Cancer (IARC) has listed BQ as group 1 human carcinogen, related with multistage progression in oral squamous cell carcinoma (OSCC). Areca nuts, Catechu, Slaked lime with betel leaf are major components of Betel quid. Nitrosamines formed from alkaloids in betel nut may be implicated in the etiology of oral cancer. OSCC are characterized by complex karyotype that involves many chromosomal aberrations (CA). The CYPs families are involved in the metabolic activation of BQ and also areca nut-specific nitrosamines. Human CYP2A subfamily members play important roles in the metabolic activation of areca nut alkaloids, which is one of major causes of OSCC. CYPs are located on human chromosome 19.

Methods: In this study cases were screened from Department of E.N.T. & Oral and Maxillofacial surgery of RKMSP Hospital, ESI Hospital, Sealdah, Kolkata and different parts of Eastern and North Eastern states of India. Blood leukocyte cultures were analyzed for mitotic index (MI). CYP2A6 gene polymorphism was studied from EDTA blood.

Results: Mitotic index are higher in cancer and pre cancer cases with chromosomal aberration (CA) than normal. Early metabolizers (EM) are susceptible to oral cancer but in case of poor metabolizers (PM) chances are less. Poor metabolizers (PM) are less prone to oral cancer due to CYP2A6 gene polymorphism.

Conclusion: Prolonged habit of betel quid and its ingredients (which act as a mood elevating food in the world) are related with oral cancer.

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cavity and leading to OSCC. The betel plant is an evergreen perennial, with glossy heart-shaped leaves, originated mainly in South and South East Asia. 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 (Leukoplakia) or reddened lesions (Erythroplakia) in the mouth that can progress to cancer. It has been found that 12.5 per cent of patients come for treatment in early stages of oral cancer.

Mitotic Index (MI) activity acts as a prognostic indicator of oral squamous cell carcinoma and this activity is higher in oral cancer cases. The Cytochrome P450 (CYP) families are divided into 14 gene families. Out of CYPs families, CYP2 and also other sub families are involved in the metabolic activation of areca nut-specific nitrosamines which is mainly trigger OSCC. Based on genetic polymorphism of Cytochrome P450 family, subjects are of two types one is (EM) Early Metabolizers and other is (PM) Poor Metabolizers. The poor metabolizers are incapable of metabolizing the exogenous compound are less prone to oral cancer.

Materials and Methods:
A case control study was conducted on all cases with cancer and precancerous, those were referred to the North Eastern India and different areas of West Bengal, Kolkata. 30 age sex matched control cases were recruited as healthy human being from camp of West Bengal. The people, who only chew betel quid except other addictions during their life style from these areas, are the main focus of the study.

A. Detailed history was taken by filling up questionnaire from all cases.
B. Leukocyte culture:
Human leukocyte culture was performed followed as the method modified from Moorhead et al. 1960. For this culture 5 ml peripheral blood samples were taken from each subject in heparinised vial under aseptic condition with the help of a sterile disposable needle. 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 subject’s duplicated culture were maintained. Leukocyte rich plasma was added to 5ml culture media supplemented with 20% fetal bovine serum and Phytohaemagglutinin M (0.04ml/ml of culture media). The cultures 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. 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 and also 100 metaphase plates were scored randomly for chromosomal aberration.

C. Molecular study:
PCR were performed with forward and reverse primer for case and control sample with 35 cycle, Sac 1 restriction enzyme is used for CYP2A6 gene polymorphism study.

Results:

Table 1: Detailed history of subjects of different places with betel quid addiction

| PLACE | NO | AGE GROUP (in years) | Addiction | BQ Addiction |
|-------|----|----------------------|-----------|--------------|
|       | Belo 30-40 | 41-50 | 51-60 | 61-70 | Abov 60 Smok ing | Alco | Betel Quid | No BQ Addiction |
| NORTH EAST CAMP 1. Assam, Karimganj | 56 | 1 | 2 | 12 | 24 | 11 | 6 | 9 | 6 | 33 | 23 |
| EASTERN INDIA CAMP 1) Bankura, Dhubai | 34 | 5 | 20 | 8 | 1 | 0 | 0 | 16 | 14 | 19 | 15 |
| 2) East Midnapur | 46 | 22 | 13 | 3 | 6 | 2 | 0 | 28 | 29 | 36 | 10 |

Table 1: Detailed history of subjects of different places with betel quid addiction
Inference: Most of the cases had betel quid chewing habit with highest percentage at 31-40 years of age.

**Table 2:** Percentage of mitotic index of studied cases and healthy control.

| Mitotic Index | Healthy Control (Mean ± SE) | Cancerous | Pre cancerous |
|---------------|-----------------------------|-----------|--------------|
|               | With betel quid (Mean ± SE) | Without betel quid (Mean ± SE) | With betel quid (Mean ± SE) | Without betel quid (Mean ± SE) |
|               | 1.31 ± 0.15                  | 3.31 ± 0.41 | 2.64 ± 1.61 | 3.03 ± 0.4 | 2.02 ± 0.74 |

Inference: In this study mitotic index are higher in cancer and pre cancer cases. Cancer and pre cancer (mainly betel quid chewing habit) cases had 2.5 folds higher mitotic index which is statistically significant ($p<0.0001^*$) are mainly related with oral cancer.

**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.

**Table 4:** Poor Metabolizers and Early Metabolizers of different Areas with betel quid chewers

| Area                                             | No of betel quid chewers | Poor Metabolizers | Early Metabolizers |
|--------------------------------------------------|--------------------------|------------------|-------------------|
| Dhulai, Bankura                                  | 19                       | 16%              | 84%               |
| Bibisanpur, East Midnapore                       | 36                       | 42%              | 58%               |
| Narrah, Bankura                                  | 24                       | 18%              | 82%               |
| Atghara, North 24 Pgs                            | 56                       | 90%              | 10%               |
| Karimganj, Assam                                 | 33                       | 60%              | 40%               |
| ENT & Oral Maxillofacial Dept. RKMSP Hospital, Kolkata | 22                       | 13%              | 87%               |

Inference: Early metabolizers are susceptible to oral cancer. It was found that the most of the pre cancer cases with betel quid chewing habit (63% in case of male and 53% in case of female) are Early metabolizers ($p<0.01$). Maximum early metabolizers (87%) are from ENT & Oral Maxillofacial Dept. RKMSP Hospital, Kolkata.

**Discussion:**
Mitotic index are higher in cancer and pre cancer cases with betel quid chewing habit than normal. Percentage of chromosomal aberration (CA) is also higher. CYP2A6 gene deletion reduces oral cancer risk in Sri Lankan population, who had betel quid chewing habit.
It was found that human CYP2A subfamily members are involved in \(N\)-nitrosamines (associated in the areca nut alkaloids) by metabolic activation, which mainly trigger CYP2A6 gene, related with Oral squamous cell carcinoma \(^{12, 13, 14}\). Poor metabolizers are less prone to oral cancer due to CYP2A6 gene polymorphism. Subjects who have polymorphism in CYP2A6 are poor metabolizers and showed band in PCR. Early metabolizers had normal CYP2A6 gene, related with Oral squamous cell carcinoma and showed no band in the molecular study. All data are statistically significant.

**Conclusion:**
In our study we have screened 311 subjects from different parts of India. Out of which more than 60\% (61.09\%) had betel quid chewing habit with higher mitotic index with CYP2A6 gene polymorphism. So, betel quid acts as a silent killer with mood elevator among Indian population.

**Conflicts of Interest**
The authors declare that they have no competing interests.

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