STUDY OF CYP2A6 GENE POLYMORPHISM AMONG BETEL QUID CHEWERS OF EASTERN AND NORTH – EASTERN INDIA

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ABSTRACT

Oral cancer is most common cancer in males and third most common in females, one of the main causative agents 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 was studied among the Eastern and North eastern Indian population. 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. Some of the cases had more than one addiction. It has been found that most of the subjects had betel quid chewing habit. Early metabolizers are susceptible to oral cancer whereas in case of poor metabolizers chances are less. Betel quid has an immense role in changing the oral pathology and developing oral cancer.

INTRODUCTION

Betel quid is fourth most commonly used psychoactive substance after tobacco, caffeine and alcohol worldwide. The betel (Piper betle) is the leaf of a vine belonging to the Piperaceae family, which includes pepper and kava. It is valued both as a mild stimulant. The betel plant originated from South and South East Asia. Many Indian people chew betel quid (a combination of areca nut, betel leaf and lime paste). Users are easily identified due to black brown teeth and stain the tongue with oral mucosa [1,2]. Betel leaf contain large amount of carcinogens known as safrole. Betel quid (BQ) products, with or without tobacco, have been classified by the International Agency for Research on Cancer (IARC) as group I human carcinogens that are associated with an elevated risk of oral potentially malignant disorders (OPMDs) [3] and cancers of the oral cavity and pharynx.

There are estimated 600 million BQ users globally. The Cytochrome p450 (CYP) families are divided into 14 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 [4].

CYPs are located on human chromosome19. The CYP2A6 gene consist of 350 kilobases located at 19q 12 – 19q 13.2 [5, 6, 7]. 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 [8]. The PM phenotype are incapable of metabolizing the exogenous compound but EM phenotype are capable of metabolizing the exogenous compound [9,10].
Our present work based on case control study suggests that the CYP2A6 genetic polymorphism has an impact on susceptibility to oral cancer.

(A) Screening of Subjects
I) Camp in Eastern India, II) Camp in North East India and III) patients attending Maxillofacial and ENT department of RKMS Hospital.

I) Eastern India camp
220 subjects were screened at a camp held in Bankura, Purba Midnapur, Atghara, 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) RKMS Hospital
2885 cases attending in one year at E.N.T OPD and Oral Maxillofacial OPD of RKMS Hospital had other complications like auditory, nasal, throat & facial problem. 35 Patient were selected for our study, 24 cases were betel quid chewers. Out of 35, 14 cases had pre-cancerous lesion, 13 cases had squamous cell carcinoma, 8 cases had pre-cancerous condition.

(B) Methods
i) Detailed history was taken from all cases by filling up questionnaire.
ii) Molecular study
PCR of different cases were performed with forward and reverse primer for case and control sample at 58°C for annealing temp with 35 cycle and total amount of PCR product is 26.5µL.

RESULTS

<table>
<thead>
<tr>
<th>PLACE</th>
<th>NO</th>
<th>AGE GROUP (in years)</th>
<th>Addiction</th>
<th>Poor metabolizer</th>
<th>Early metabolizer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Below 30 31-40 41-50 51-60 61-70 Above 70</td>
<td>Smoking Alcohol Betel Quid No BQ Addiction Tea Drinker Non Tea Drinker</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NORTH EAST CAMP 1. Assam, Karimganj</td>
<td>56</td>
<td>1 2 12 24 11 6</td>
<td>9 6 33 23 40 16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EASTERN INDIA CAMP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1) Bankura, Dhubli</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2) East Midnapur, Bibhisanpur</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3) North 24 Pgs, Atghara</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4) Narrah, Bankura</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RKMS Hospital</td>
<td>35</td>
<td>2 7 8 11 7 0</td>
<td>20 8 24 11 29 6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td>311</td>
<td>66 73 64 65 32 11</td>
<td>114 65 190 121 265 46</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: Some cases had more than one addiction.

<table>
<thead>
<tr>
<th>AREA</th>
<th>NO OF BETEL QUID CHEWERS</th>
<th>POOR METABOLIZER</th>
<th>EARLY METABOLIZER</th>
</tr>
</thead>
<tbody>
<tr>
<td>Karimganj, Assam</td>
<td>33</td>
<td>60%</td>
<td>40%</td>
</tr>
<tr>
<td>Dhubli, Bankura</td>
<td>19</td>
<td>16%</td>
<td>84%</td>
</tr>
<tr>
<td>Bibisnapur, East Midnapore</td>
<td>36</td>
<td>42%</td>
<td>58%</td>
</tr>
<tr>
<td>Atghara, North 24 Pgs</td>
<td>56</td>
<td>90%</td>
<td>10%</td>
</tr>
<tr>
<td>RKMS Hospital</td>
<td>22</td>
<td>13%</td>
<td>87%</td>
</tr>
<tr>
<td>Narrah, Bankura</td>
<td>24</td>
<td>18%</td>
<td>82%</td>
</tr>
</tbody>
</table>

Early metabolizers are susceptible to oral cancer where as in case of poor metabolizers chances are less.

DISCUSSION
Oral cancer is one of the leading cancers in most Asian countries [11]. India has largest betel quid consuming population in the world. The habit of chewing betel quid is due to low cost, easily available and also as a mood elevator in the day to day busy scheduled life. CYP2A6 gene deletion reduces oral cancer risk in betel quid chewers in Sri Lanka [12]. Poor metabolizer are less prone to oral cancer than early metabolizer due to CYP2A6 gene polymorphism. Subjects who have polymorphism in CYP2A6 are poor metabolizer and showed band in PCR.
Early metabolizer had normal CYP2A6 gene and showed no band in PCR.

**CONCLUSION**

The substances which are present in the betel quid not only have the cytotoxic, mutagenic property but it also involved in genetic, enzymatically and molecular mechanism in the development of carcinogenesis. In our study it has been found that more than 50% cases from North Eastern states were poor metabolizer, whereas more than 50% cases of Eastern region (except North 24 Pgs) were early metabolizer.

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**REFERENCES**