Journal Homepage: - www.journalijar.com
INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)
Article DOI: 10.21474/IJAR01/9912
DOI URL: http://dx.doi.org/10.21474/IJAR01/9912
ISSN NO. 2320-5407

RESEARCH ARTICLE

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

Aniket Adhikari[1] and Madhusnata De[2].

1. M.Sc., Research Scholar, Vivekananda Institute of Medical Sciences (V.I.M.S).
2. M.Sc., PhD Professor, Department of Genetics .Ramakrishna Mission Seva Pratishthan (RKMSP), 99, Sarat Bose Road. Kolkata – 700026, India.

Manuscript Info

Manuscript History
Received: 14 August 2019
Final Accepted: 16 September 2019
Published: October 2019

Key words:-
Betel Quid (BQ), Oral Squamous Cell Carcinoma (OSCC) , Chromosomal Aberration (CA), Mitotic Index (MI), Early Metabolizers (EM), Poor Metabolizers (PM).

Abstract

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 chromosome19. **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.

Introduction:-

There is highly adverse relationship between Oral squamous cell carcinoma and Betel Quid (BQ). Oral Squamous Cell Carcinoma (OSCC) is one of the most common malignancies in South and South East Asian countries. Oral pre malignancies are also very common in betel quid chewers (BQ) and about 10% of these undergo malignant transformation. In the year2020-2025,the percentage of OSCC are rises more than 70% [1] .Oral cancers account for the third highest cancer related mortality among men aged 30-69 in Indian population [2]. The betel quid is made up of areca nut, catechu, slaked lime and often tobacco, which are placed in a betel leaf in various parts of India. Chewing betel quid releases carcinogenic nitrosamines from the areca nut that can cause pre neoplastic changes of the oral

Corresponding Author:- Aniket Adhikari.
Address:- M.Sc., Research Scholar, Vivekananda Institute of Medical Sciences (V.I.M.S).

cavity and leading to OSCC [3].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 [4].The Cytochrome P450 (CYP) families are divided into14 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 [5]. Based on genetic polymorphism of Cytochrome P450 family, subjects are of two types one is (EM) Early Metabolizers and other is (PM) Poor Metabolizers [6]. The poor metabolizers are incapable of metabolizing the exogenous compound are less prone to oral cancer [7, 8].

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 [9, 10]. 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
		Belo 30	31-40	41-50	51-60	61-70	Abov 70	Smok ing	Alco l	Betel Quid	No Addiction
NORTH EAST CAMP 1. Assam, Karimganj	56	1	2	12	24	11	6	9	6	33	23
EASTERN INDIA	34	5	20	8	1	0	0	16	14	19	15
CAMP	46	22	13	3	6	2	0	28	29	36	10
1) Bankura,Dhulai	89	28	18	21	15	6	1	27	3	56	33
2)East Midnapur,	51	8	13	12	8	6	4	14	5	22	29

Bibhisanpur 3) North 24 Pgs, Atghara 4)Narrah, Bankura											
ENT & Oral Maxillofacial Dept. RKMSP, Kolkata.	35	2	7	8	11	7	0	20	8	24	11
TOTAL	311	66	73	64	65	32	11	114	65	190	121

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 [11].

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.

Acknowledgements:-

The authors are thankful to Prof (Dr.) Madhusnata De with Secretary, Ramakrishna Mission Seva Pratishthan, Kolkata for kind permission to use the laboratory, Dr. P.B.Kar and all doctors are related with this work.

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