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GST & CYP Polymorphism Related to Tea Drinking and Oral Pathology

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ABSTRACT Individual cancer susceptibility is the result of several host factors, including differences in life-style habits and genetic susceptibility. There is a correlation between CYP1A1 polymorphism (*Msp I*) and oral cancer susceptibility. Individuals carrying the deletions of GSTM1 and GSTT1 are at high risk of developing oral cancers. Again an increased risk of oral carcinoma is indicated by an increased micronuclei frequency. In the present study on tribal and non-tribal population of Assam, CYPm2m2 genotypes were found in about 14.05% cases having leukoplakia and other oral problems. In the same cases GSST null genotypes were found to be 24.35%. In comparison to the controls the micronuclei frequencies were found to be one fold increased for the cases. In spite of having heavy exposure to the carcinogens due to tobacco chewing and *bidi* smoking, regular tea drinking is decreasing the risk of developing cancer to very little extent.

INTRODUCTION

Oral cancer is one of the leading cancers in most Asian countries (Lin et al. 2002). Epidemiological studies has revealed that in addition to tobacco and alcohol, betel nut exposure is the most potent risk factor for oral squamous cell carcinoma in Taiwan (Scully et al. 2000).

Apart from the effects of external chemical carcinogens, risk of developing different types of cancer is dependent on the inter-individual variability in sensitivity towards carcinogen. Metabolic activation of most chemical carcinogens is refined by Phase I enzymes (cytochrome P-450) and detoxification by conjugation via the various Phase II enzymes like Glutathione–S-transferase, N-acetyl transferase etc. Therefore the coordinated expression and regulation of Phase I and Phase II drug metabolizing enzymes and their metabolic balance may be an important host factor in determining whether exposure to carcinogens results in cancer or not. Polymorphisms in the genes that code for these enzymes may alter expression or function, thus increasing or decreasing the activation or detoxification of carcinogenic compounds (Sato et al. l999).

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Molecular epidemiological studies have proved that an individual can be susceptible to leukoplakia and cancer both environmentally and genetically. Cytochrome P450s (CYPs) are mostly the Phase I enzymes in the activation pathway. CYP1A1, one of the various forms of CYP enzymes is considered to play an important role in the activation of polyaromatic hydrocarbons (PAHs) such as Benzo-∝-pyrene (BP)[CYP], Glutathione-S-transferases [GSTs], a group of Phase II enzymes in detoxification pathway detoxify many electrophilic substrates by conjunction with reduced glutathione. Four members of the GST genes-GSTM1, GSTT1, GSTP1 and GSTM3 display polymorphisms that have been associated with increased risks for certain cancers. Absence of GSTM1 activity a GST enzyme that detoxifies the reactive metabolites of BP and other PAHs is due to homozygous inherited deletion of the gene. A similar deletion polymorphism in the GSTT1 gene, encoding a theta-class enzyme is also known. GSTT1 metabolizes various potential carcinogens such as monohalomethanes and ethylene oxide present in tobacco smoke and some endogenously generated reactive products derived from lipid peroxides. Various studies examining GST and CYP genotypes as determinants for oral leukoplakia and cancer risks have been described (Nair et al. 1999)

The human enzyme CYP1A1, which is well conserved is involved in the activation of major

classes of procarcinogens like PAHs and aromatic amines and is present in many epithelial tissues. About 10% of the Caucasian population has a highly inducible form of the CYPA1 enzyme (termed B [A] P-hydroxylase or previously aryl hydrocarbon hydroxylase), which is associated with an increased risk for bronchial, laryngeal and oral cavity tumors in smokers. Studies on the association of the genetic polymorphism of CYP1A1 high inducibility phenotype and polymorphism of the MspI restriction site. The CYP1A1 Ile-Val mutation in the heme-binding region results in a two-fold increase in microsomal enzymes activity. It is in complete linkage disequilibrium in Caucasians with the CYP1A1 MspI (m1) mutation, which has also been associated with increased catalytic activity (Landi et al. 1994).

The formation of micronuclei in eukaryotic cell is taken as the end point of chromosomal damage or segregation errors (Geard and Chen 1990). The presence of micronuclei reflects a genotoxic or carcinogenic exposure. Due to its association with chromosomal aberrations, micronuclei have been used since 1937 as an important marker of genotoxic exposure and MN assay becomes effective in metaphase analysis. (Heddle et al. 1983). The MN test, and consequently the repair index, might be useful for monitoring the clinical evolution of subjects with healed or surgically removed tumors or leukoplastic lesions, after chemo- or radiotherapeutic treatments, by means of intra- and inter-individual cellular comparisons (Ramirez et al. 2002).

Tea is considered as a source of strong antioxidants. Tea polyphenols have been found to have inhibitory actions on tumor induction. Tea polyphenols are known as catechins and approximately presents in 30-42% of the dry weight in the solid brewed green tea. Polyphenols from black tea are theaflavins and thearubigins. Antioxidant properties of tea have been partially resulted in several health benefits. (Wiseman et al. 1997; Rice-Evans 1999) Monomeric green tea polyphenols have chemopreventive properties. A study suggests that black-tea derived polyphenols also show one of the chemo-preventive properties shown by green tea polyphenols. Initiation is the starting phase of carcinogenesis. At this time Polymeric black tea polyphenol fractions hamper the formation of [3 H]-B (a) P-derived DNA adducts as well as the activity cytochrome P450 isozymes CYP1A1 and 1A2 *in vitro* employing rat liver microsomes (Krishnan and Maru 2005).

The present study was carried out by the Thalassaemia Research Unit of Ramakrishna Mission Seva Pratishthan, Kolkata in collaboration with the Department of Anthropology, Dibrugarh University, Dibrugarh. The typical population of the North-Eastern India bears the combinations of contradictory life-style habits of both tobacco intake and tea drinking. So the genotypes and the life-style history of this ethnic population were of great interest.

MATERIALS AND METHODS

Study Cases: A study of genetic polymorphisms was performed from 2004-2006 amongst the tribal and non-tribal populations of Assam.The tribal groups were Sonowal, Deoris, Ahom, Chakma, Apatani, Chutia, Kamti, Koch,Mising etc.All participating cases were given questionnaire to have informations regarding their life-style history. Informations were obtained about their frequency of consumption of tea either in raw form or with milk and prevalence of oral diseases like oral ulcers, leukoplakias and other white lesions in mouth. History of tobacco intake was also noted.

Control Participants: 51 controls were chosen randomly from healthy individuals of the same population without any prior diagnosis of oral problems.

Cytogenetic Assay: The subjects were told to rinse their mouths with water and oral smears were taken on clean microscopic SLIDES. Smears after air-drying, were fixed in 80% methanol. Slides were stained with Giemsa solution and MN frequencies were scored. Same person scored 1000 cells blindly in each case to determine the MN percentage (Sarto *et al.*1990).

DNA Isolation: Blood was drawn from the subjects in a fresh EDTA vial and genomic DNA was extracted from from frozen peripheral blood leukocytes by using Quiagen DNA extraction kit.

Genotyping Assay: All results were confirmed by repeated experiments.

CYP1A1 Gene: Genotypic identification of CYP1A1 gene was carried out by PCR amplification followed by digestion with MspI enzyme. This detects the substitution of CCGG for CTGG in the MspI site. The PCR comprises of 30 cycles of following conditions: 1 min. denaturation at

Table 1: Characteristics of tea-consumption in the study population

	Consumption of black tea (% of intake)				*Consumption of tea with milk (% of intake)*			
	Never	*Occasional*	*1-4 cups/ day*	*>4 cups/ day*	*Never*	*Occasional*	*1-4 cups/ day*	*>4 cups/ day*
Contrl population	4.1	3.9	19.5	5.8	2.2	5.8	54.9	3.9
Cases	0.1	2.7	33.2	8.8	2.3	4.6	51.2	7.1

95°C; 1 min primer annealing at 57°C; 1 min primer extension at 72°C.Amplified fragments were digested with MspI for 2-4 hrs at 37°C.Digested products were electrophoresed in 2% agarose gel. (Sato et al.1999). Genotypes of all individuals were noted[m1m1=1078bp; m1m2=1078,878, 200bp and m2m2=878,200bp] (Sikdar et al. 2003).

GST Gene: GSTM1 (220bp) and GSTT1 (450bp) portions were amplified together with β-globin(268bp) and were analyzed after electrophoresis in 2% agarose gel. (Sreelekha et al. 2001). Absence of 220bp or 450bp portions implied GSTM1 or GSTT1 null genotypes. Subjects with all fragments were the non-null genotypes.

Sample size was too low for carrying out the statistical analysis for each individual tribal.

RESULTS

144 cases participated in the study. The cases and the controls were more or less similar in ethnicity. All were from low-income group and their nutritional intake was same. Though they were not supposedly exposed to environmental or occupational toxic hazards, they all had strong tobacco habits. All of them were found to have tobacco habits with betel chewing Simultaneously they also had tea-drinking habit with different frequencies.

Among 144 cases and 51 controls the mean age was 54 and 50 years respectively.71% of controls and 69% of cases were female.98% of the cases and 95% of the controls were found to be from the tribal origin. Controls were involved in heavier black tea drinking (>4 cups/day) than the cases (Table 1).

People had oral problems of different types, including leukoplakias. Though the cases of minor oral problems like stained teeth, oral infections were excluded from the study, the cases were mostly affected with common oral pathology (Table 2). Leukoplakia was found to develop in the cases with less frequent tea-drinking habits. So, it is evident that tea polyphenols cannot result in protecting any kind of oral pathology.

Table 2: Description of the population having oral problems and their tea-habits

Oral problems	*Consumption of tea (% of intake)*
Leukoplakia (26%)	18
Oral ulcers (24%)	22
Benign white lesions (19%)	22
Other oral problems like gingivitis, pyorrhea, gum bleeding etc. (31%)	38

The distributions of age, duration of tobacco habit and tea consumption in years as well as the frequencies MN per 1000 cells per individual were scored. The controls showed the normal range of micronuclei distribution in the exfoliated buccal cells. Among 37 cases having pre-cancerous lesions the percentage of micronuclei frequency in average was found to be 3.2 ± 0.873.Around one-fold increase was found in the micronuclei frequency of the cases in comparison to the controls (Table 3).

Table 3: Oral pathology status by study of micronuclei

Types of subjects	*No. of subjects*	*% of MN (avg)*
Control	51	0.28±0.012
Cases	144	0.31±0.015

Genotypic assay was confirmed by repeating the experiments twice. Though CYPm2m2 genotypes were found in some cases (Table 4) of 14.05%, the frequencies of distribution of CYPm1m2 genotype were not of very significant. Leukoplakia cases were found to have more

Table 4: Unadjusted means (+ SE) of CYP and GST genotypes and tea-intake in the population

	N (Mean ± S.E)	
	Consumption of black tea	*Consumption of tea with milk*
Total Population	55	89
GSTM1 Wild	13.7 ±1.4	14.2±1.2
GSTM1 Null	10.2±1.8	9.9±1.1
GSTT1 Wild	15.3±1.6	8.2±1.2
GSTT1 Null	9.8±1.6	10.9±1.8
CYPm1m1	16.0±2.7	9.9±1.7
CYPm1m2	9.7± 0	7.7±6.0
CYPm2m2	8.3±4.6	15.1±2.1

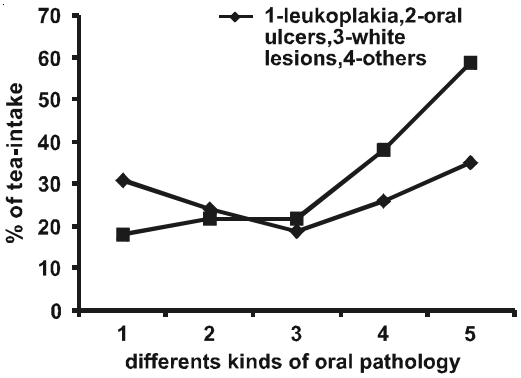

Fig. 1. Demography of Developing Oral Pathology

average distribution of CYPm2m2 genotypes (2.2% more with respect to the controls). GST null genotypes were significantly found in leukoplakia cases (29 out of 37 cases). The distribution of GSTM1 null was more frequent in comparison GSTT1 genotypes.

DISCUSSION

As the cases and controls were chosen from the more or less same ethnical and nutritionally same background, the effects of all the related factors was thought to be same in all respects.

An elevated DNA and mutational damage associated with 'at risk' alleles has been found in both Asian and Caucasian smokers. Large-scale studies on combination of CYP1A1 variants and GSTM1 null genotype in an ethnicity could predict elevated head and neck cancer risk (Bartch et al. 2000). From the present study it is evident that distribution of CYP1A1m2m2 genotypes and simultaneously GSTM1 and GSTT1 null genotypes was different from the normal controls. To study the effects of combination of genotypes of both CYP and GST polymorphisms on development of oral pathology and tea- intake would require more sample size. The effect of GSTM1 present or deleted is more noticeable among heavy tobacoo chewers and light tea drinkers in comparison to heavy tobacco chewers and heavy tea drinkers.

Interaction of CYP1A1 and GSTM1 null genotypes provide some suggestive leads. Future efforts in this regard should emphasize on more gene-environment interactions. (Olshan et al. 2000)

During the malignant process, the path to carcinogenesis appears to be correlated with increasing MN frequencies. This idea is supported by the similar MN frequencies found in the controls, as well as by the investigations of Stich and Rosin (1983). Different from what was expected, none of the frequencies of MN and other multinucleated cells were significantly correlated with age among patients and controls as has often been reported (Kayal et al. 1993). A significant difference was found between micronuclei frequency of the controls and the cases denoting higher risk of oral carcinoma.

Metabolism of carcinogens under genetic control is an important factor in modulating individual susceptibility to cancer. Information on susceptibility to cancer is valuable for identifying high-risk individuals, allowing for early diagnosis and reduction of risk exposure to carcinogens.

In conclusion it can be said that the studied population is bearing a significant fraction of risky genotypes for developing cancers. Heavy tobacco habit is likely to increase the risk. The tea drinking habits showed to have an association with not developing oral lesions in some cases. The future study should involve several genetic polymorphisms and environmental carcinogens having different susceptibility levels on for developing cancer in larger population groups.

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