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ASSOCIATION OF CYTOCHROME CYP2C9 GENE POLYMORPHISM IN CARCINOMA OF OROPHARYNX AND HYPOPHARYNX

Oncology

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ABSTRACT

This study was carried out to investigate the association of cytochrome P450 2C9 (*CYP2C9*) gene polymorphism with squamous cell carcinoma (SCC) of oropharynx & hypopharynx. One hundred ten males suffering from locally advanced squamous cell carcinoma and an equal number of healthy controls were genotyped for *CYP2C9*2* and *CYP2C9*3*, leading to poor metabolizers (PMs) by PCR-based RFLP. The frequency of genotypes of *CYP2C9*2* (40%) and *CYP2C9*3* (16%) were found to be significantly higher in the HNSCC controls as compared to the cases. The data suggests a significant association of the *CYP2C9* polymorphism with SCC of oro & hypopharynx and underlining the importance of genotyping in determining the risk of occurrence of SCC of oropharynx & hypopharynx

KEYWORDS

CYP2C9 gene, gene polymorphism, HNSCC

INTRODUCTION

Squamous cell carcinoma of the head and neck (SCCHN) takes the 5th place in cancer incidence worldwide[1]. Overall, head and neck cancer accounts for more than 500,000 cases annually worldwide[2-4]. Majority of neoplasms (90%) of head and neck are squamous cell carcinomas (HNSCC) that develop in the squamous layer of the mucosal lining, in upper aero digestive tract. Males are affected significantly more than females with a ratio ranging from 2:1 to 4:1.

Tobacco chewing, smoking, betel quid chewing, areca nut, HPV infection and alcohol consumption are the most important causative factors in development of SCCHN. Approximately 57% of men & 10% of women worldwide are tobacco users & its prevalence is increasing [5]. Epidemiological and human genetic studies have identified different types of population "at risk," one consisting of individuals with heavy exposure to carcinogens, and the other consisting of carriers of cancer-predetermining germ-line mutations in genes, that because of high penetrance, confer a very high risk for cancer per se. There is also another group of predisposing polymorphic, low penetrance genes. Because of individual differences in susceptibility to develop a tobacco smoke–related cancer, only a small percentage of them will ultimately suffer from SCCHN, however, the mechanisms are still unclear[6-8].

Tarun have reported that the compressive strength of rubberized concrete can be improve when fine aggregate was fully replaced by fine crumb rubber. He also indicated that if the rubberParticles have rougher surface or given a pretreatment, the better and improved bonding may develop with the surrounding matrix, and that may result in higher compressive strength.

This threat of harmful compounds is encountered by the Phase I and Phase II biotransformation enzymes that exist in the epithelial cells lining the aero digestive tract which activate and detoxify them, thus modifying risk[9-11]. The fact that the variant genotypes of cytochrome P450 1A1 (CYP1A1), CYP1B1 and null genotypes of GSTM1 and GSTT1 are associated with an increased risk of HNSCC who were regular tobacco users suggests a gene-gene and gene-environment interaction[12-14].

The official name of this gene CYP2C9 is "cytochrome P450, family 2, subfamily C, polypeptide 9."CYP2C9 is the gene's official symbol. CYP2C9 encodes a member of the cytochrome P450 superfamily of enzymes. The cytochrome P450 proteins are monooxygenases which catalyze many reactions involved in drug metabolism and synthesis of cholesterol, steroids and other lipids.CYP2C9 is known to be involved in the metabolism of some of the antineoplastic drugs such as cyclophosphamide, etoposide, tamoxifen, and ifosfamide. CYP2C9 enzyme metabolizes several carcinogenic and mutagenic substrates including heterocyclic aromatic amines and polycyclic aromatic hydrocarbons (PAHs)[15].Genetic polymorphism has also been reported for CYP2C9. Variant mutant (both homozygous and heterozygous) CYP2C9*2 and CYP2C9*3 genotypes account for 'poor-metabolizer' (PM) phenotype resulting in slow metabolism of drugs and other substrates metabolized by CYP2C9. An increased frequency of CYP2C9*2 allele in the patients with lung cancer has been reported. Variant alleles of CYP2C9 were reported to increase the risk of distal colorectal adenoma [16]. Thus , this case control study was planned to study the association of polymorphism in cytochrome P450 2C9 (CYP2C9) with occurrence of squamous cell carcinoma (SCC) of oropharynx and hypopharynx .

MATERIALS AND METHODS

A case control study was conducted at department of radiotherapy, King George's Medical University, KGMU), Lucknow, India. Male cases suffering from cancer oropharynx & hypopharynx and visiting the OPD of Radiotherapy Department of KGMU from July 2015 to July 2016 were included in this study. The study group comprised 100 cases with oropharynx and hypopharynx squamous cell carcinoma and equal number of matched healthy controls. The cases had squamous cell carcinoma of the oropharynx and hypopharynx which was confirmed by cytopathological or histopathological examinations and were advised a combination treatment of chemoradiotherapy. All the cases included in the study belonged to the same ethnic group (Indo-European community) of North India based on geographical location and linguistic basis. Controls were frequency-matched to cases by year of birth in 10-year classes. It was ensured that the controls also belonged to the same geographical location and socio-economic conditions. Based on medical check-up, controls were not found to suffer from any chronic disease.

Informed consents of the cases & controls were obtained before inclusion in the study. All patients were staged according to AJCC 2010 recommendations. Genomic DNA was isolated from blood samples collected from patients as well as controls. Polymorphisms were identified by PCR technique using RFLP .Specific restriction enzymes and primers were used. The statistical analysis was performed with the SPSS software package v22.o. Standard chi square tests were carried out to determine whether genotype or allele frequency of carcinogen metabolizing enzymes among the cases and controls are in Hardy Weinberg equilibrium.

Previously untreated subjects belong to same ethnic group of north India.with age between 18-65 years, histologically proven Squamous cell carcinoma of oropharynx and hypopharynx without any history of previous malignancy. Patients of nasopharyngeal carcinoma.patients with prior treatment in the form of chemotherapy, radiotherapy or chemoradiation.patients having KPS<70,Non tobacco user i.e. lack of exposure.Malignancy other than cancer of oropharynx and hypopharynx were excluded from the study Ethanol was obtained from Bengal Chemicals, India, Dimethyl sulfoxide (DMSO), Agrose powder, boric acid crystal and tris buffer were obtained from SISCO Research Laboratories Pvt. Ltd (SRL), Mumbai, India.10X buffer, MgCl2, dNTPs, Forward Primer, Reverse Primer, Taq Polymerase for PCR amplifications were obtained from MBI Fermentas, Germany. EDTA (Ethylene Diamine Tetra Acetic Acid) was obtained from Ranbaxy, New Delhi. Genomic DNA isolation kit was obtained from QIAGEN, Genetix. Restriction Enzyme and its buffer obtained from MBI Fermentas, Germany. For quality control, 10%of the samples were selected randomly and re-genotyped to confirm the authenticity of the results obtained earlier, and they were found to be in 100% agreement.

For studying the polymorphisms in CYP2C9 genes, blood was obtained from controls individuals. Informed consent was taken from the individuals before sampling. A detailed questionnaire including health states, ethnic origins etc. of the individuals were also filled. 5 ml of blood were drawn from healthy individual in the tube containing ACD solution, an anticoagulant and stored at 40C till processed for isolation of genomic DNA.

RESULTS

Controls were frequency-matched to cases by year of birth in 10-year classes. Pearson's chi-square test used to analyse distribution of the cases and controls on the basis of age. The age wise distribution was similar in both the groups. (Table 1)

The mean age was found to be 50.3± 9.4 years among cases whereas in controls it was 48.4 ± 9.6 years. Maximum cases 36 (36%) and controls 34 (34%) were of 51-60 years age group bracket. Lowest number of the cases 3 (3%) and controls 4 (4%) were from 21-30 year age group. Non-parametric chi square test was used to analyse distribution of the cases on the basis of cancer stage. (Table 2).

TABLE 1. AGE DISTRIBUTION OF STUDY POPULATION

Age group (in years)	Cases		Controls		$\chi 2$	p-value
	Number	Percentage	Number	Percentage		
21-30	3	3.0	4	4.0	1.14	.88
31-40	17	17.0	18	18.0		
41-50	30	30.0	34	34.0		
51-60	36	36.0	34	34.0		
>60	14	14.0	10	10.0		
Mean	50.3± 9.4		48.4 ± 9.6		.91	.16

Table2. DISTRIBUTION OF CASES ON THE BASIS OF CANCER STAGE

Stage	Number of patients	Percentage	$\chi 2$	p-value
II	17	17.0	17.4	**<.001**
III	51	51.0		
IV	32	32.0		

TABLE 3 GENOTYPICDISTRIBUTION IN STUDY POPULATION

Genotype	Groups				$\chi 2$	p-value
	Cases		Controls			
	Number	%	Number	%		
CYP2C9-1	44	44.0	1	1.0	63.3	**<.001**
CYP2C9-2	40	40.0	63	63.0		
CYP2C9-3	16	16.0	36	36.0		

Lowest number of the cases 17 (17%) were enrolled in stage II. Highest 51 (51%) patients belonged to stage III, 32 (32%) cases were recruited in stage IV. Genotype distribution was analysed using Fisher's exact test. (table 3)

In cases maximum percentage of genotype were found to be of CYP2C9-1 genotype (44.0%) followed by CYP2C9-2 (40.0%) and CYP2C9-3(16.0%). In controls, CYP2C9-2 type genotype was present in 63% subjects, followed by 36% of CYP2C9-3 genotype and 1% CYP2C9-1genotypes. Genotype distribution was significantly different in cases and controls ($p<0.05$).

DISCUSSION

CYP2C9 encodes a member of cytochrome p450 superfamily of enzymes. It is involved in the detoxification of several tobacco smoke-derived carcinogens as well as in metabolism of some antineoplastic drugs. To date, there are at least 33 variants of CYP2C9 (*1B through to *34) being identified. CYP2C9*2 and CYP2C9*3 differ from the wild-type CYP2C9*1 by a single point mutation. CYP2C9*2 which codes for amino acid change Arg144Cys and CYP2C9*3 is responsible for amino acid change Ile359Leu. [17] Indian population has a unique structure, genetically more homogenous than conglomerate populations, and also show similarity in life style and dietary habits which may enable greater success in genomic and epidemiological studies. The frequencies of CYP2C9*2 in Indian population have found to be between 3–5 % whereas CYP2C9*3 ranges from 4–8 % [18-20] CYP2C9 variant alleles are associated with altered metabolism of alkylating agents that are well established mutagens. [21] An association has also been reported between high levels of bronchial bulky DNA adduct with CYP2C9 genotypes in lung cancer cases with increased formation of bronchial bulky DNA adduct in cases withCYP2C9*2 alleles compared to those with CYP2C9*3. [22]

It is therefore possible that the variant genotypes of CYP2C9 (*2 and *3) may modulate susceptibility to smoking-induced cancers. Due to the high conservation of the genetic code through generations, such a mutation can be inherited. However, if a certain mutation is seen in more than 1% of a population group, the variation is considered a polymorphism. Polymorphisms usually occur as point mutations where a single (or a few) base(s) is substituted (substitution), deleted (deletion) or an extra base is inserted (insertion). These point mutations are commonly known as single nucleotide polymorphisms (SNPs). Genetic polymorphism has also been reported for CYP2C9. Results of the present study have demonstrated that CYP2C9 variants modulate the susceptibility to SCC of oropharynx and hypopharynx.

In CYP*2 polymorphism wild type base pair CC(c = cytosine) is replaced either CT (t = thymine) heterozygous mutant type or TT homozygous mutant type. In CYP*3 polymorphism AA (adenine) wild type base pair is replaced by either heterozygous AC or homozygous CC mutant base pair. Variant mutant (both homozygous and heterozygous) CYP2C9*2 and CYP2C9*3 genotypes account for 'poor-metabolizer' (PM) phenotype resulting in slow metabolism of drugs and other substrates metabolized by CYP2C9.An increased frequency of CYP2C9*2 allele in the patients with lung cancer has been reported.[23-25]

The mean age was found to be 50.3± 9.4 years among cases whereas in controls it was 48.4 ± 9.6 years.The distribution was similar in both the groups and hence the p-value was >.05 being statistically insignificant. This result shows that most of the population suffering from SCC of oropharynx and hypopharynx are middle aged.Most of the cases enrolled in the study were advanced and belonged to stage III (51%) or stage IV (32%) while stage II forming 17% of the cases. Likewise, an increase in risk in cases of drinking alcohol withCYP2C9 PM genotypes has suggested that alcohol possibly interacts with CYP2C9 genotypes in increasing the risk to HNSCC.

Polycyclic aromatic hydrocarbons are known to be generated both during cigarette smoking and tobacco chewing. The increased risk observed in cases using tobacco with PM genotypes of CYP2C9 could be explained by higher affinity of CYP2C9 for benzo (a) pyrene.

The present study has demonstrated that a several fold increase in the risk to SCC of oropharynx and hypopharynx in the cases with variant genotypes (PMs) of CYP2C9. We have come to the conclusion that CYP2C9 genotypes may have a role in interacting with environmental risk factors in modifying the susceptibility to SCC of oropharynx and hypopharynx. This is a very preliminary study and further studies with larger sample size may establish this preliminary result in future and the genetic polymorphism of CYP2C9 may play an important role in hypopharynx and oropharynx cancer susceptibility.

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