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EFFECT OF DRUG METABOLISING ENZYME CYTOCHROME CYP2C9 GENE POLYMORPHISM ON TREATMENT RESPONSE IN CARCINOMA OF OROPHARYNX AND HYPOPHARYNX

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ABSTRACT This study was carried out to investigate the association of polymorphism in cytochrome P450 2C9 (*CYP2C9*) with response in patients receiving chemoradiotherapy. One hundred males suffering from locally advanced squamous cell carcinoma were genotyped for *CYP2C9*2* and *CYP2C9*3*, leading to poor metabolizers (PMs)by PCR-based RFLP. Each case was assessed thoroughly for treatment response following WHO criteria. Cases assessed for response carrying variant alleles of both *CYP2C9*2* (46.0%) or *CYP2C9*3* (22.0%) were found to respond poorly to the radio-chemotherapy. The data suggests a significant association SCC of oropharynx and hypopharynxand treatment outcome with the *CYP2C9* polymorphism and underlining the importance of pre-therapeutic genotyping in determining the treatment modality.

KEYWORDS : CYP2C9 gene, gene polymorphism, HNSCC

INTRODUCTION

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[1-3]. 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[4-6].

The administration of chemotherapy along with RT is commonly referred to as concurrent chemoradiation or simply chemoradiation. This approach is so far the standard of care in most locally advanced cancers of the oropharynx & hypopharynx based on the results of multiple randomized trials that have documented a survival benefit. Chemoradiation appears to confer a survival benefit over RT alone in both the "unresectable" setting as well as the postoperative setting. In general, most trials of concurrent chemoradiation have not documented reductions in the rates of distant metastases with the addition of concurrent chemotherapy to RT. As a result, the survival benefit imparted by chemotherapy is primarily due to improvements in local control.[7,8]

Chemotherapy also has many systemic toxic effects. Thus, now there has been great interest in developing assays that can be used as biomarkers of the extent and persistence of effects caused by exposure to toxic agents. Lymphocytes have known advantages for use in the development of non-invasive assays to screen human population for toxicant exposure and have been shown to express several members of CYP gene family, whose protein products are involved in the oxidative metabolism of a wide variety of drugs and chemicals.[1]

The newer methods of molecular analysis to detect DNA polymorphisms, such as polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) techniques may make genotyping screening approaches increasingly feasible which drug(s) will suit to a particular individual can be predicted based upon phenotype and/or genotype studies of relevant drug-metabolising enzyme(s). The identification and cloning of all the major human drug-metabolizing P450 enzymes, and the major gene variants that cause inter-individual variability in drug response has now provided the basis for the use of predictive pharmacogenetics to yield therapies that are more efficient and cost effective. Thus, this study was planned to study the genetic polymorphism in CYP2C9 gene in patient of cancer oropharynx and hypopharynx and to analyse effect of CYP2C9 gene polymorphism on treatment response to chemoradiation.[9]

MATERIALS AND METHODS

This study was conducted at department of radiotherapy, King George's Medical University, KGMU), Lucknow, India. A total of 100 male, aged between 18-65 years, previously untreated, histologically proven Squamous cell carcinoma of oropharynx and hypopharynx without any history of previous malignancy visiting the OPD of Radiotherapy Department of KGMU from July 2015 to July 2016 were included in this study. 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 with KPS 70 and above, adequate bone marrow reserve Hb> 10 gm %, WBC>4000/cu mm, platelet count >100000/cu mm, normal renal, cardiac, liver and lung function, no other malignancy should be present, history of exposure to tobacco i.e. cigarettes (≥100) , cigars/cigarillos/filtered little cigars (≥50),regular pipes(≥50),water pipes/hookahs(≥1), chewing tobacco(≥20), e-cigarettes(≥1),snus(≥1) and dissolvable tobacco products(≥1) and surgically unresectable patients and or surgically resectable patients not willing for surgery. Defaulter patients, patients of nasopharyngeal carcinoma, prior treatment in the form of chemotherapy, radiotherapy or chemoradiation, patients having KPS<70, on tobacco user i.e. lack of exposure and malignancy other than cancer of oropharynx and hypopharynx were excluded from the study. Informed consents of all the cases were obtained before inclusion in the study. All study subjects completed a questionnaire covering medical history. Genomic DNA was isolated from blood samples collected from patients. Polymorphisms were identified by PCR technique using RFLP .Specific restriction enzymes and primers were used. [10] Standard treatment was given chemoradiotherapy (Total dose of 70Gy in 35#, in 7 weeks with concurrent cisplatin 35mg/m2 weekly).Response was categorized as complete response(CR), partial response(PR), progressive disease(PD) or no change(NC) based on WHO assessment criteria . [11]CR & PR were classified as responders and PD and NC as non responders.The statistical analysis was performed with the SPSS software package v22.o. Standard chi square tests were carried out to determine genotype or allele frequency of carcinogen metabolizing enzymes among the cases Patients were treated by Co60 (Bhabhatron, BARC Mumbai/ Theratron 780e,Ottawa,Canada.) for radiotherapy planning of the patients was done on x-ray simulix evolution simulator, Siemens.

For studying the polymorphisms in CYP2C9 genes, blood was

obtained from all cases. 5 ml of blood were drawn from healthy individual in the tube containing ACD solution, an anticoagulant and stored at 4^0C till processed for isolation of genomic DNA. Isolation of DNA and RFLF was done as standard protocol.

RESULTS

The mean age was found to be 50.3± 9.4 years in these cases. Genotype distribution was analysed, (table) 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%).(table 1) Non-parametric chi square test used to analyse and compare distribution of responders to treatment. More than half of the cases were found to be non-responders (57%) while only 43% responded to chemoradiation, but it was not a significant difference (p=0.16) in percentage distribution between responders and non responders . (table 2) Table 3 shows the relationship between genotypic variation and treatment response. Fisher's exact test was used to find the association. It revealed that maximum of the respondents were proportionately high in CYP2C9-1 group whereas maximum of the non- respondents were significantly high in the genotype CYP2C9-2 & 3 group and this association was statistically significant (p<.05).

Table 1. Genotypic Distribution In Study Population

Genotype		
	Cases	
	Number	%
CYP2C9-1	44	44.0
CYP2C9-2	40	40.0
CYP2C9-3	16	16.0

Table 2. Distribution Of Responders to Treatment

Response to treatment	Number of patients	Percentage	χ2	p-value
Responders	43	43.0	1.96	.16
Non-responders	57	57.0		

Table 3. Relationship Of Genotype With Prognosis

Genotype CYP2C9	Response to Chemoradiation				χ2	p-value
	Responders		Non-responders			
	Number	%	Number	%		
1	24	55.8	20	35.1	11.0	**.001**
2	11	.29.7	29	46.0		
3	2	5.4	14	22.2		

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

Polymorphic CYP2C9 also play a role in metabolism of drugs used in chemotherapy, which may cause variability in drug response.[12,13] This study was carried out to investigate the influence of genetic polymorphisms in CYP2C9 on modulating the treatment outcome in the cases of squamous cell carcinoma of oropharynx and hypopharynx.

The data of the present study has shown that functionally important polymorphism of *CYP2C9* exists in North Indian population. The frequency of the variant genotypes (*CYP2C9*1/*2* and *CYP2C9*3*) was found to be higher (40% and 16%, respectively)than that reported in South Indian (7% and 1%)population. This could be partly attributed to the population structure of India comprising a mixture of endogamous ethnic groups.

More than half of the cases were found to be non-responders (57%) while only 43% responded to chemoradiation. One of the reason of this may be the advance stage of presentation.

Most of the responders were proportionately high in CYP2C9-1 group whereas maximum of the non- respondents were significantly high in the genotype CYP2C9-2 & 3 group and this association was statistically significant(p<.05).

Since CYP2C9 is involved in the detoxification of carcinogenic substrates, the increased risk observed in cases with PM genotypes of CYP2C9 in our study could be attributed to their poor detoxifying ability. Our study has shown that chemotherapeutic response is modified in patients with PM genotypes of CYP2C9. A higher percentage of non-responders were observed in the cases with PM genotypes of CYP2C9. Furthermore it is also possible that PMs of CYP2C9 can modify the treatment outcome in cases receiving chemoradiotherapy.

This is a very preliminary study and further studies with larger sample size may establish these preliminary result in future and the genetic polymorphism of CYP2C9 may play an important role in hypopharynx and oropharynx cancer treatment response.

In recent times, many studies have stimulated from a candidate gene-based pharmacogenetic approach to genome-wide pharmacogenomic analyses to identify biomarkers for selection of patient-tailored therapies. In coming days, multi-gene panel platform will be utilized to optimize drugs use and characterize the individual genetic background.[14] These strategies not only increase the efficacy and safety of therapeutic products but also inform us that why some patients respond well to a drug, and some experience adverse reactions, while others do not**.** So FDA recommend that Pharmacogenomic information may be provided in drug or biological product labeling to inform health care providers about the impact of genotype on response to a drug through description of relevant genomic markers, functional effects of genomic variants, dosing recommendations based on genotype, and other applicable genomic information. [15]

Likewise in treatment of Type 2 diabetes mellitus, human genomic information predict the personalized response to drug therapy. Inter-individual variability in response to oral antidiabetic drugs is due to polymorphisms in genes encoding drug receptors, transporters, and metabolizing enzymes are actively involved in glycemic/HbA1c management of metformin. Pharmacogenetic studies provide insights on the relationship between individual genetic variants and variable therapeutic outcomes of various oral antidiabetic drugs. Pharmacogenetics therefore, is a step towards personalized medicine which will greatly improve the efficacy of treatment of chronic and complex deaeses.[16]Pharmacogenomic studies of Severe cutaneous adverse reactions have also made important steps, as the deterrence of these adverse reactions. [17]

Recently scientists assessed the pharmacogenomic biomarkers allelic varieties in 18 European populations by studing 1,931 pharmacogenomics biomarkers in 231 genes. Data showed significant inter-population pharmacogenomic biomarker allele frequency differences. These data reflect differences in the prevalence of high-risk genotypes in these populations. This approach may consequently add to considerable cost-savings in the healthcare costs and demonstrates to be cost-effective.[18] The cost-effectiveness of targeted treatments depends on many aspects together with prevalence of biomarkers, sensitivity and specificity, expenditures of the test. Assessment studies should be done to protect and improve the cost-effectiveness comparability of stratified drug therapies in formulating national and international standards. [19]On the other hand there are few unanswered and in addressed fields. Gene maturation varies between paediatric and adult age groups, and adult pharmacogenomic details may not be effective in paediatric population. In view of disparities in physiological maturation and gene expression between paediatric and adult age groups, assessments studying pharmacogenomic properties exclusively in paediatric age groups should be accomplished whenever important biomarkers are presented.[20]

The Pharmaco Genomic Mutation Database is a wide-ranging manually selected pharmacogenomics record. Two most important adequately peer-reviewed literature sources of Pharmaco Genomic Mutation Database data are Food and Drug Administration and European Medicines Agency. These Pharmaco Genomic Mutation Database curators collect information on precise genomic spot, disease

details, sequence modifications, on resulting phenotype, drugs, specific patient population, study design, context, statistical significance and other relevant characteristics of pharmacogenomic variants. Variants are described into functional categories on the basis of their effect on efficacy, pharmacodynamics, pharmacokinetics and clinical outcome. [21]Significant progress has been done in learning effects of genetic polymorphisms of P450s on pharmacokinetics, and it has assisted the development of optimized pharmacotherapy**.** [22] Whole exome sequencing has the prospective of identifying new findings that may be predictive of poor pharmacotherapy effects. [23] Sharing pharmacogenetic results from corner to corner clinical settings and electronic health documentation is a critical footstep for the implementing clinical pharmacogenetics. Standardized pharmacogenetic terms will advance the perceptive and interpretations of pharmacogenetic results. It will decrease misinterpretation by preserving constant nomenclature. [24] Thus we need comprehensive and extensive efforts to detect new and distinct biomarkers associated with chemoradiation and other drug therapies in different populations. This will reduce cost and side effects of the treatment along with improvement in treatment outcome of complex diseases.

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