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Research Article

# EFFECT OF POLYMORPHISMS IN DRUG METABOLISING ENZYME CYTOCHROME P4502A6 IN TREATMENT RESPONSE IN HEAD AND NECK SQUAMOUS CELL CANCER PATIENTS

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**ABSTRACT**

A case control study was carried out to investigate the association of functionally important polymorphism in cytochrome P450 2A6 (CYP2A6) genes with head and neck squamous cell carcinoma (HNSCC) and treatment response in cases receiving a combination of chemo- radiotherapy. The study group consisted of 300 males suffering from HNSCC and an equal number of male controls. Multivariate logistic regression analysis revealed the frequency of the variant alleles of CYP2A6 were significantly higher in the controls when compared to the cases with the history of tobacco use. Also, the frequency of the deletion variant alleles of CYP2A6*4C were significantly higher in the controls when compared to the cases with the history of alcohol consumption. These showed that the variant polymorphisms of CYP2A6 decreased the risk of HNSCC. Interestingly, only 37% of the cases carrying the variant forms of CYP2A6 responded to the treatment for HNSCC when compared to those with wild-type genotype (79%). Thus, it can be said that polymorphisms in CYP2A6 not only modified the risk to HNSCC but also played a major role in determining the chemotherapeutic response.



## 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%) are head and neck 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 causes in developing 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].

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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].

Three major classes of carcinogens, Polycyclic Aromatic Hydrocarbons (PAHs), Tobacco Specific Nitrosamines (TSNAs), and aromatic amines, play important roles in tobacco-associated cancers [15]. Areca nut, a major component of Betel Quid, contains certain alkaloids that give rise to nitrosamines, some of which such as N-nitrosoguvacoline, 3-methylnitrosamino-propionitrile, 3-methylnitrosamino propionaldehyde and N-nitrosoguvacine, are shown to be carcinogenic & act as an etiologic factor for leukoplakia, oral submucous fibrosis and consequently HNSCC. The major areca nut alkaloids are arecoline, arecaidine, arecolidine, guvacoline and guacine. Reactive oxygen species are generated in the oral cavity during Betel Quid chewing due to the addition of slaked lime [$Ca(OH)_2$] into Betel Quid. It acts as a solvent for other carcinogens or generates or exacerbates coincident inflammation, producing significant Reactive Oxygen Species[16].

CYP2A6 cytochrome P450, family 2, subfamily A, polypeptide 6 encodes a member of the cytochrome P450 super family of enzymes. Human CYP2A6 is regulated via nuclear factor-erythroid 2 related factor 2 (Nrf2). CYP2A6 is expressed in the liver lung, trachea, nasal mucosa, and sex organs such as breast. CYP2A6 is directly induced by oestrogen in an ER alpha-dependent manner, implying a biological role of CYP2A6 in oestrogen-responsive tissues. This mechanism can also explain clinical aspects of increased nicotine metabolism under oestrogen-rich environments. The psychoactive compound responsible for tobacco addiction, nicotine and the potent carcinogens are metabolized by CYP2A6 (formerly CYP2A3)[17]. Individuals with certain allelic variants are said to have a poor metabolizer phenotype, meaning they do not efficiently metabolize coumarin or nicotine. This gene is part of a large cluster of cytochrome P450 genes from the CYP2A, CYP2B and CYP2F subfamilies on chromosome 19q13.2.

CYP2A6 has over 35 different alleles with additional subgroups. Star (*) nomenclature is used to describe different CYP2A6 alleles, with the "wild type" reference allele defined as *1. Genetic variations include whole and partial gene deletions, single nucleotide polymorphisms.CYP2A6 genotypes are often classified into predicted phenotype groups, describing the effect on enzyme activity, for example 'poor', 'slow', 'intermediate', 'normal'/ 'extensive', or 'ultra rapid'/ 'fast' metabolizer. CYP2A6*1B has greater nicotine clearance when compared to *1A. SNPs of CYP2A6 *2, *4, *7, *9, *10, *12 and *17 show reduced clearance of nicotine. Genetic variants in the CYP2A6 gene can result in reduced expression by affecting transcriptional or translational processes[18]**.** Genetic variations in CYP2A6 gene or genotypes associated with reduced synaptic serotonin activity may influence the success of smoking cessation treatment. CYP2A6 genotype conferring lower enzyme activity may be advantage for individuals undergoing chemotherapeutic treatment for neoplastic disease because reduced detoxification potentially enhances effectiveness of cytotoxic drugs. Anticancer that have been shown to be substrates for CYP2A6 are, for example, tegafur, SM-12502,5-fluoro-uracil metabolites and steroids. Cisplatin is still one of the most potent anti-neoplastic agent & its activity is mainly based on the covalent modification of the DNA molecule. The causes of nephrotoxic and neurotoxic effects of cisplatin include generation of reactive oxygen species, which may lead to oxidative damage, lipid peroxidation, and apoptosis. There were earlier indications that platinum based drugs may modulate the levels mRNA levels of CYP.

Cisplatin inhibits the CYP2C9, CYP2B6, CYP2A6 and CYP1A2 activities, however, the decrease is not greater than 25% at the highest concentration of the drug, although the interaction is much more evident for transplatin which is clinically ineffective[19].

***Survey of Literature***

Interindividual and interethnic differences have been reported for CYP2A6. Of several functionally important polymorphisms in CYP2A6, CYP2A6*1B and CYP2A6*4 are prevalent in the Asian population (Chinese and Japanese) when compared to the Caucasians[20]. As most of the functionally important polymorphic alleles of CYP2A6 either result in abolished activity (*2, *4, *5 and *20) or reduced activity (*6, *7, *10, *11, *12, *17, *18 and *19), CYP2A6 genetic variation could play an important role in tobacco-related cancer risks [21]. Though most of the studies reported a decreased risk to tobacco induced cancers such as lung cancer and HNSCC among individuals carrying the CYP2A6*4 deletion allele, inconsistencies exist in the literature. As compared to the Caucasian and Oriental populations, not much data is available on the distribution of variant allele frequencies of CYP2A6 & its association with HNSCC risk in Indian Population The present case control study was therefore initiated to investigate the association of polymorphisms in CYP2A6 (CYP2A6*1B and CYP2A6*4C) with treatment outcome in HNSCC in North Indian population and also the interaction of CYP2A6 polymorphism with HNSCC risk factors like tobacco use and alcohol consumption.

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.

Recently 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[22,23]

## MATERIALS AND METHODS

A hospital based case control study was conducted at King George's Medical University (KGMU), Lucknow, India to investigate the association of polymorphism in cytochrome P450 2A6 (CYP2A6) with HNSCC treatment outcome in cases receiving chemo-radiation. The study group comprised 300 males suffering from HNSCC visiting OPD facility of Radiotherapy Department, KGMU from 2012 to 2016. The cases had squamous cell carcinoma of Head & Neck which was confirmed by cytological or histopathological examinations and were advised a combination treatment of concurrent chemo-radiotherapy. 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. An equal number of matched healthy individuals (n = 300) of the same ethnic group (Indian-European community) of North India and drawn from the same geographical and linguistic lineage were also included in the study and served as the controls. For collecting the blood samples of controls, health camps were organised in Lucknow and neighbouring districts and sampling was done. Based on the routine check-up, only those healthy controls were included in the study. The protocol for research work was approved by the human ethics committee and it conforms to the provisions of the declaration of Helsinki (1995). Informed consent was obtained from the study subjects for inclusion in the study and before the collection of blood samples and it was also ensured that the subject anonymity was preserved. All study subjects completed a questionnaire

covering medical, residential and occupational history. Information about dietary habits, family history of disease and tobacco use (tobacco chewing and smoking) was also obtained.

The following study design was adopted:

1. Informed consent of the patient was taken in format as approved by the research cell, K.G.M. University, Lucknow.
2. The history of the patient with special inquiry about predisposing factors was taken.
3. General and systemic examination was done.
4. Local examination was done including the primary disease and neck examination.
5. All patients underwent routine investigations such as Haemogram. Liver Function Test, Kidney Function Test, Chest X-ray PA view. Special investigations such as Direct laryngoscopy and CT/MRI scan were done as & when required.
6. All patients were staged according to AJCC 2010 recommendations.
7. 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.

Primer used was

CYP2A6 FP -5'CACCGAAGTGTTCCCTATGCT-3' **(21Bases)** (**PRODUCT SIZE-1322bp)**
CYP2A6 RP-5'TGTAAATGGGCATGAACGCCC-3' **(21Bases)(Chr. No- 19)**

8. Standard treatment was given chemoradiotherapy (Total dose of 70Gy in 35#, in 7 weeks with concurrent cisplatin 35mg/m2 weekly).
9. Response was categorized as complete response (CR), partial response(PR), progressive disease(PD) or no change(NC) based on WHO assessment criteria[24]. CR & PR were classified as responders and PD and NC as non responders.
10. Toxicity was graded according to RTOG toxicity criteria[25].
11. 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.
12. Patients were treated by Co60 (Bhabhatron, BARC Mumbai/ Theratron 780e, Ottawa, Canada.) for radiotherapy.
13. Planning of the patients was done on x-ray simulix evolution simulator, Siemens.

***Selection Criteria***

***Inclusion Criteria***

1. Previously untreated, histologically proven squamous cell carcinoma of head and neck.
2. KPS 70 and above.
3. Adequate bone marrow reserve Hb> 10 gm %, WBC>4000, platelet count >100000.
4. AGE 18-65 yrs.
5. Normal renal, cardiac, liver, lung function.
6. No cancer other than head and neck cancer.
7. HISTORY of EXPOSURE to tobacco (cigarettes (≥100 times), cigars (≥50 times), regular pipes (≥50 times), water pipes/hookahs (≥1 time), chewing tobacco (≥20 times), e-cigarettes (≥1 time), snus (≥1 time), and dissolvable tobacco products (≥1 time)[26].
8. Consenting patient.
9. Surgically unresectable patients or surgically resectable patients not consenting for surgery.
10. All patients should belong to same ethnic group of north India.

***Exclusion Criteria***

1. Patient having any concurrent illness with head and neck cancer or history of any prior malignancy.
2. Age <18 or>65 years
3. Defaulter patient.
4. Prior treatment in the form of chemotherapy, radiotherapy or chemoradiation.
5. Diagnosed case of Nasopharyngeal Carcinoma.
6. Non tobacco user.i.e lack of exposure.
7. Non consenting patient.
8. Surgically resectable patients consenting for surgery i.e. stage I & II without adverse pathological findings on post-operative HPE(close/positive margins, lymphovascular invasion, perineural invasion & extracapsular extension).

***Dna Isolation & Genotype Analysis***

One millilitre of blood was collected into citrate containing tubes from all the subjects. DNA was isolated from whole blood with the Flexi Gene DNA kit (Qiagen, CA) following the manufacturer's protocol. Isolated DNA was subsequently used for genotyping studies. The method of Ariyoshi *et al* [27] was followed for determining the *CYP2A6* genotypes. The PCR reaction mixture in 50_l contained 0.2_M of each primer, 0.3mMdNTPs, 2.5mMMgCl2, 0.75U Taq DNA polymerase (MBI, Fermentas, Germany) and 100 ng DNA. PCR conditions were initial denaturation at 94 ◦C for 5min followed by 35 cycles of 94 ◦C for 1 min, 62 ◦C for 1min, 72 ◦C for 2.0 min and final extension at 72 ◦C for 10 min. The PCR products of 1322 bp length were digested at 37 ◦C with two restriction enzymes *Bsh12361* and *Eco81I* (MBI Fermentas, Germany) and the fragments of digested PCR products were analysed by 2% agarose gel stained with ethidium bromide[28] .The reaction mixture in 25_l contained 0.5_M of each primer, 0.2mM dNTPs, 1.5mM MgCl2, 0.75U Taq DNApolymerase (MBI, Fermentas, Germany) and 100 ng DNA. PCR condition was initial denaturation at 94 ◦C for 5 min followed by 35 cycles of 94 ◦C for 30 s, 60 ◦C for 1min, 72 ◦C for 1.5min and final extension at 72 ◦C for 10 min. Sterilewater was substituted for genomic DNA and served as a negative control in each PCR reaction. Amplified products were then digested with 8 units of restriction enzyme *Alw261* (New England Biolabs, UK) for overnight at 37 ◦C. The fragments of digested PCR products (wildtype: 176 bp; polymorphic heterozygous: 176 bp, 91 bp and 85 bp; and homozygous variant: 91 bp and 85 bp) were analysed by 8% PAGE stainedwith ethidium bromide.

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.

## RESULTS

The mean ages of the controls and cases were 49.45 ± 9.7and 50.07 ± 8.8 years, respectively (Table 1).

**Table 1** Age Distribution

| Age in years | Cases (n=100) | | Controls (n=100) | | Fisher's exact value@ | p-value |
|---|---|---|---|---|---|---|
| | No. | % | No. | % | | |
| 18-35 | 30 | 10.0 | 27 | 9.0 | 2.086 | 0.55 |
| 36-45 | 60 | 20.0 | 60 | 20.0 | | |
| 46-55 | 135 | 45.0 | 123 | 41.0 | | |
| 56-65 | 75 | 25.0 | 90 | 30.0 | | |
| Mean ± SD | 50.07 ± 8.8 | | 49.45 ± 9.7 | | t-value# =0.82 | 0.41 |

@- Fisher's exact test used;#- Independent t-test used; p-value <0.05 is significant;

By tumor site, a majority of cases were suffering from oral cancer (39%) followed by oropharyngeal (29%) and laryngeal cancers (21%) (Table 2).

Most of the cases were grouped into locally advanced HNSCC (85%) with a small percentage presenting as stage II HNSCC (15%) (Table 3)

**Table 2** Sitewise Distribution

| Site | Cases | | Non-parametric $\chi 2$ | p-value |
|---|---|---|---|---|
| | No | % | | |
| Oral cavity | 117 | 39.0 | 54.7 | **<.001** |
| Oropharynx | 89 | 29.7 | | |
| Hypopharynx | 30 | 10.0 | | |
| Larynx | 64 | 21.3 | | |

Non-parametric chi-square test used;p-value <.05 is significant;

**Table 3** Stagewise Distribution

| Stages | Cases | | Non-parametric $\chi 2$ | p-value |
|---|---|---|---|---|
| | No | % | | |
| Stage I | 0 | 0.0 | 46.5 | **<.001** |
| Stage II | 45 | 15.0 | | |
| Stage III | 135 | 45.0 | | |
| Stage IV | 120 | 40.0 | | |

Non-parametric chi-square test used;p-value <.05 is significant;

Higher prevalence of tobacco use in the form of smoking and tobacco chewing & Alcohol consumption among cases resulted in a significantly enhanced HNSCC risk, when compared to the controls.(Table 4)

**Table 4** Distribution of Risk Factors Among Cases & Controls

| Addiction | Cases (n=300) | | Controls (n=300) | | $\chi 2$ | p-value |
|---|---|---|---|---|---|---|
| | No. | % | No. | % | | |
| Tobacco use | 300 | 100.0 | 156 | 52.0 | 189.4 | <.001 |
| Alcohol use | 230 | 76.7 | 98 | 32.7 | 117.1 | <.001 |

Pearson chi-square test used;p-value <.05 is significant;

The genotype distribution of the polymorphic *CYP2A6* in controls & cases is shown in Table 5.

As evident from Table 5, the genotype frequencies of *CYP2A6* for the heterozygous *CYP2A6*4C* (**1A*/**4C)* & (**1B*/**4C*) in cases & controls were found to be in Hardy–Weinberg equilibrium. The frequency of genotypes with one or both deletion alleles (**4C*) of *CYP2A6* (**1A*/**4C, *1B*/**4C, *4C*/**4C*) was significantly higher in controls when compared to the cases (p value <0.001, 95% C.I.=0.12-0.39) , showing a marked decrease in HNSCC risk. No such association was found with *1A & *1B variants which were somewhat equally distributed among cases and controls.

The effects of the interaction of the risk modifiers i.e. tobacco & alcohol use in the form of cigarette smoking and tobacco chewing with variant genotypes of *CYP2A6* is summarized in Table 6 & 7 respectively.

As evident from Table 6, the frequency of the variant alleles of CYP2A6 (*1A/non-*1A + non-*1A/non *1A)(p <0.001,95% C.I.=0.27-0.65) &(*1A/*4C + *1B/*4C + *4C/*4C,95% C.I.=0.07-0.29) were significantly higher in the controls when compared to the cases with the history of tobacco use either in the form of smoking or chewing. This conferred a significantly decreased risk of development of HNSCC.

As evident from Table 7, the frequency of the Deletion variant alleles of CYP2A6*4C (*1A/*4C + *1B/*4C + *4C/*4C, p <0.001,95% C.I.=0.06-0.33) were significantly higher in the controls when compared to the cases with the history of alcohol consumption. This association was not observed in non-alcoholics. This conferred a significantly decreased risk of development of HNSCC.

Table 8 shows the correlation between the treatment response in cases and the genotypes. In cases with homozygous wild genotype (*1A*1A) the frequency of responders was high (78.8%) compared to non-responders (21.2%). In case of variant genotypes, the proportion of non-responders (63.1%) was significantly higher than the responders(36.9%) (p <0.001,95% C.I. = 0.09-0.26), particularly in the deletion variants (*4C), both homozygous or heterozygous (p<0.001,95%C.I.=0.03-0.29). This clearly depicts a poor treatment response in variant genotypes on CYP2A6.

## DISCUSSION

The study revealed that the frequency of conversion type allele (*1B) was 31.6% which was similar to what is observed in Caucasian population but lower when compared to the Chinese & Japanese counter part[20]. Likewise, the frequency of the deletion type allele (*4C) was 11.8% which was similar

**Table 5** Distribution of Polymorphism of CYP2A6 Amongst Cases & Controls

| Polymorphism of CYP2A6 | Cases (n= 300) | | Controls (n=300) | | Odds Ratio | 95%CI | | p-value |
|---|---|---|---|---|---|---|---|---|
| | No. | % | No. | % | | Lower Limit | Upper Limit | |
| *1A/*1A | 132 | 44.0 | 96 | 32.0 | 1.0 (Ref) | - | - | - |
| *1A/*1B | 114 | 38.0 | 105 | 35.0 | .79 | .54 | 1.1 | .21 |
| *1B/*1B | 35 | 11.7 | 36 | 12.0 | .71 | .41 | 1.2 | .20 |
| *1A/*4C | 12 | 4.0 | 34 | 11.3 | .25 | .12 | .52 | <.001 |
| *1B/*4C | 6 | 2.0 | 21 | 7.0 | .21 | .08 | .53 | .001 |
| *4C/*4C | 1 | 0.3 | 8 | 2.7 | .09 | .01 | .73 | .02 |
| 1A/non-1A + non-*1A/non *1A | 168 | 56.0 | 204 | 68.0 | .59 | .42 | .83 | .003 |
| *1A/*4C + *1B/*4C +*4C/*4C | 19 | 6.3 | 63 | 21.0 | .21 | .12 | .39 | <.001 |

Uni-variate logistic regression analysis used; Ref denotes the reference group;In the outcome variable, control group is taken as comparable group; p-value<.05 is significant;

between CYP2A6 polymorphism and alcohol consumption

**Table 6** Gene (CYP2A6) Environment (Tobacco) Interaction With HNSCC Risk

| Polymorphism of CYP2A6 | Cases (n= 300) | | Controls (n=156) | | Odds Ratio | 95%CI | | p-value |
|---|---|---|---|---|---|---|---|---|
| | No. | % | No. | % | | Lower Limit | Upper Limit | |
| **Tobacco users (n=456)** | | | | | | | | |
| *1A/*1A | 132 | 44.0 | 39 | 25.0 | 1.0 (Ref) | - | - | - |
| *1A/non-*1A + non-*1A/non*1A | 168 | 56.0 | 117 | 75.0 | .42 | .27 | .65 | **<.001** |
| *1A/*4C + *1B/4C + *4C/*4C | 19 | 6.3 | 37 | 23.7 | .15 | .07 | .29 | **<.001** |

Uni-variate logistic regression analysis used; Ref denotes the reference group;In the outcome variable, control group is taken as comparable group; p-value<.05 is significant;

**Table 7** Gene (CYP2A6) Environment (Alcohol) Interaction With HNSCC Risk

| Polymorphism of CYP2A6 | Cases (n= 230) | | Controls (n=98) | | Odds Ratio | 95%CI | | p-value |
|---|---|---|---|---|---|---|---|---|
| | No. | % | No. | % | | Lower Limit | Upper Limit | |
| **Alcohol users (n=328)** | | | | | | | | |
| *1A/*1A | 127 | 55.2 | 24 | 24.4 | 1.0 (Ref) | - | - | - |
| *1A/non-*1A + non-*1A/non *1A | 103 | 44.7 | 74 | 75.5 | .26 | .15 | .44 | **<.001** |
| *1A/*4C + *1B/*4C + *4C/*4C | 14 | 6.0 | 18 | 18.3 | .14 | .06 | .33 | **<.001** |

Uni-variate logistic regression analysis used; Ref denotes the reference group;In the outcome variable, control group is taken as comparable group; p-value<.05 is significant;

| Polymorphism of CYP2A6 | Cases (n= 70) | | Controls (n=202) | | Odds Ratio | 95%CI | | p-value |
|---|---|---|---|---|---|---|---|---|
| | No. | % | No. | % | | Lower Limit | Upper Limit | |
| **Alcohol non-users (n=272)** | | | | | | | | |
| *1A/*1A | 5 | 7.1 | 72 | 35.6 | 1.0 (Ref) | - | - | - |
| *1A/non-*1A + non-*1A/non *1A | 65 | 92.8 | 130 | 64.3 | 7.2 | 2.7 | 18.6 | **<.001** |
| *1A/*4C + *1B/*4C + *4C/*4C | 5 | 7.1 | 45 | 22.2 | 1.6 | .43 | 5.8 | .47 |

Uni-variate logistic regression analysis used; Ref denotes the reference group;In the outcome variable, control group is taken as comparable group; p-value<.05 is significant;

**Table 8** Treatment Response In Cases Of HNSCC With Polymorphism of CYP2A6

| Genotype | Cases (n=300) | | Responders (n=166) | | Non-responders (n=134) | | Odds Ratio | 95%CI | | p-value |
|---|---|---|---|---|---|---|---|---|---|---|
| | No. | % | No. | % | No. | % | | Lower | Upper | |
| *1A/*1A | 132 | 44.0 | 104 | 78.8 | 28 | 21.2 | 1.0 (Ref) | - | - | - |
| *1A/Non *1A+Non*1A/Non *1A | 168 | 56.0 | 62 | 36.9 | 106 | 63.1 | .15 | .09 | .26 | **<.001** |
| *1A/*4C+*4C/*4C+*1B/ *4C | 19 | 6.3 | 5 | 26.3 | 14 | 73.7 | .09 | .03 | .29 | **<.001** |

Uni-variate logistic regression analysis used; Ref denotes the reference group;In the outcome variable, Non-responders group is taken as comparable

to that found in oriental population, much lower than the Japanese population(20-31%) & much higher than the Caucasian counterpart (0.5-4%) [20,29,30]. The frequency of the wild allele (*1A) was 65.1% & 60.8% in cases and controls respectively.

The increase in frequency of variant genotypes, either homozygous or heterozygous for *4C , among controls when compared to cases indicates that polymorphism in CYP2A6 is associated with altered risk of HNSCC. The lowering of risk attributed to the *4C variant might be due decreased activation of several carcinogens by CYP2A6 including TSNAs. Further is has also been shown that variant genotypes are more likely to be light smokers than those with wild genotypes who are more likely to be heavy smokers. This further reduces the HNSCC risk in variant genotypes. It has been seen that the urinary excretion of cotinine closely relates with the level of activity of CYP2A6, with individuals harbouring *4C allele having several fold or absent excretion [31,32]. No association was found between HNSCC risk and *1B allele in our study. Various other studies have also shown similar result [30,33]. A significant association was also seen with history of alcohol consumption. This might be due to the additive or synergistic effect of alcohol with tobacco in upper aerodigestive tract cancers. There has not been any study linking polymorphism of CYP2A6 with altered alcohol metabolism.

Our study has further depicted that polymorphism in CYP2A6 can alter the treatment response to concurrent chemoradiation. The treatment was poor in the variant genotypes particularly those who were carrying a deletion subtype allele and the wild type (*1A/*1A) genotype responded extremely well to treatment. Though not much is known about the role of *CYP2A6* in the metabolism of cisplatin, it may modulate the levels mRNA levels of CYP2A6.

Cisplatin inhibits the CYP2C9, CYP2B6, CYP2A6 and CYP1A2 activities, however, the decrease is not greater than 25% at the highest concentration of the drug, although the interaction is much more evident for transplatin which is clinically ineffective [19].

Poor treatment response in the variant subtypes could thus be attributed to an altered metabolism of cisplatin, decreased bioavailability and efficacy

## CONCLUSION

In summary, a reduced risk for HNSCC was observed in cases with variant *CYP2A6* genotypes. Our data showed that this reduced risk was associated with *CYP2A6 *4C* allele. We further showed a reduction in HNSCC risk in tobacco users with variant *CYP2A6* genotypes. Further, poor treatment response of concurrent chemoradiation in cases with variant genotypes of *CYP2A6* have demonstrated the role of polymorphisms in these genes in predicting an individual's response to cisplatin based chemoradition, and thus, may help in designing a suitable or alternate concurrent chemotherapy regimen. With the dawn of pharmacogenomics and personalized medicine , it is time that we look at genetics to provide us with the answers that we are looking for, especially in oncology & we shift from the mindset of 'one-drug-fits-all' to 'the right drug for the right patient at the right dose and time.'

***Future Scope***

To our knowledge, this is the first time we have demonstrated that the CYP2A6 polymorphisms are associated with the response to radiotherapy with concurrent cisplatin based chemotherapy in HNSCC. A larger study will be necessary to validate our data that suggest that the CYP2A6 polymorphism may influence the response and clinical outcome of HNSCC patients to radiotherapy with concurrent cisplatin based chemotherapy, together with functional studies to establish its mechanistic basis.

We propose that the CYP2A6 polymorphism should be routinely done to select patients who are more likely to benefit from treatment with cisplatin based concurrent chemo-radiotherapy in HNSCC if results of our study are validated on larger patient population.

Several limitations of our study must be acknowledged. Firstly, the sample size is this study was small, and it may have limitation to be generalized especially when correlating HNSCC risk with factors like tobacco chewing, smoking and alcohol intake. Secondly, Measuring clinical response and time to progression may be critical to elucidate further mechanism by which CYP2A6 polymorphisms affects outcome. These parameters can distinguish whether CYP2A6 polymorphisms are predictive of treatment response or are prognostic by determining outcome. On the basis of the expected outcome of the patient, both predictive and prognostic factors may be important in the choice of chemotherapeutic regimens. Thirdly, CYP2A6 is not the only gene that affects the treatment outcome in HNSCC. Others like XRCC, ERCC, CYP2C9, CYP1A1 etc also affect the treatment outcome via their own pathway & their expression and polymorphism must be taken into account.

We further intend to do a follow up study to include a larger sample size to get meaningful results which can be more aptly and correctly applied to the general patient population of northern India.

## Refferences


1. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. *CA Cancer J Clin* 2005; 55: 74-108.
2. Jemal A, Bray F, Center MM, *et al*. Global cancer statistics. *CA Cancer J Clin* 2011; 61:69.
3. Chaturvedi AK, Anderson WF, Lortet-Tieulent J, *et al*. Worldwide trends in incidence rates for oral cavity and oropharyngeal cancers. *J ClinOncol* 2013; 31:4550.
4. Ferlay J, Shin HR, Bray F, *et al*. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer* 2010; 127:2893.
5. Hecht SS. Cigarette smoking: cancer risks, carcinogens, and mechanisms. *Langenbecks Arch Surg* 2006; 391: 603-13.CrossRef, PubMed
6. Hecht SS. Tobacco carcinogens, their biomarkers and tobacco-induced cancer. *Nat Rev Cancer* 2003; 3: 733-44.CrossRef,PubMed
7. Wogan GN, Hecht SS, Felton JS, Conney AH, Loeb LA. Environmental and chemical carcinogenesis. *Semin Cancer Biol* 2004; 14:473-86.CrossRef, PubMed
8. Ames BN. Dietary carcinogens and anticarcinogens. Oxygen radicals and degenerative diseases Science 1983; 221: 1256-64. CrossRef,PubMed
9. de Waziers I, Cugnenc PH, Yang CS, Leroux JP, Beaune PH. Cytochrome P 450 isoenzymes, epoxide hydrolase and glutathione transferases in rat and human hepatic and extrahepatic tissues. *Pharmacol ExpTher* 1990; 253: 387-94.PubMed
10. M. Hashibe, P. Brennan, R.C. Strange, R. Bhisey, I. Cascorbi, P. Lazarus, M.B. Oude Ophuis, S. Benhamou, W.D. Foulkes, T. Katoh, C. Coutelle, M. Romkes, L. Gas-pari, E. Taioli, P. Boffetta, Meta- and pooled analyses of GSTM1, GSTT1, GSTP1 and CYP1A1 genotypes and risk of head and neck cancer, Cancer Epidemiol. *Biomarkers Prev*. 12 (2003) 1509-1517.
11. P. Brennan, P. Boffetta, Mechanistic considerations in the molecular epidemiology of head and neck cancer, IARC Sci. Publ. 157 (2004) 393-414.
12. A.P. Singh, P.P. Shah, M. Ruwali, N. Mathur, M.C. Pant, D. Parmar, Polymorphism in cytochrome P4501A1 is significantly associated with head and neck cancer risk, Cancer Invest., in press.
13. A.P. Singh, P.P. Shah, N. Mathur, J.T.M. Buters, M.C. Pant, D. Parmar, Genetic polymorphisms in cytochrome P4501B1 and susceptibility to head and neck cancer, *Mutat. Res.* 639 (2008) 11-19.
14. M. Singh, P.P. Shah, A.P. Singh, M. Ruwali, N. Mathur, M.C. Pant, D. Parmar, Association of genetic polymorphisms in glutathione S-transferases and susceptibility to head and neck cancer, *Mutat. Res.* 638 (2008) 184-194.
15. Kawajiri K (1999). "CYP1A1". IARC Scientific Publications (148): 159-72.PMID 10493257.
16. Hashibe M, Boffetta P *et al*: Evidence for an important role of alcohol- and aldehyde-metabolizing genes in cancers of the upper aerodigestive tract. *Cancer Epidemiol Biomarkers Prev*, 15: 696-703, 2006
17. W. Gene ID: 1548
18. McDonagh Ellen M, Wassenaar Catherine, David Sean P, Tyndale Rachel F *et al* Pharmacogenetics and genomics. 2012
19. VlastimilMasek *et al*. Interaction of antitumor platinum complexes with human liver microsomal cytochromes P450. *Anti-Cancer Drugs* 2009, Vol 20 No 5

20. C. Xu, S. Goodz, E.M. Sellers, R.F. Tyndale, CYP2A6 genetic variation and potential consequences, *Adv. Drug Deliv. Rev.* 54 (2002) 1245-1256.
21. T. Kamataki, M. Fujieda, K. Kiyotani, S. Iwano, H.Kunitoh, Genetic polymorphism of CYP2A6 as one of the potential determinants of tobacco- related cancer risk, *Biochem. Biophys. Res. Commun.*338 (2005) 306-310.
22. C.C Harris, H. Autreep, R. Conner, L.A. Barret, E.M. Mc Dowell, B.F. Trump, interindividual variation in binding of benzo(a)pyrene to DNA in cultured human bronchi, *Science* 194(1976) 1067-1069.
23. J.P. VandenHeuvel *et al*, CYP1A1 mRNA level as a human exposure biomarker. 2003- 2006.
24. Weber JL. Human DNA polymorphisms and methods of analysis. *CurrOpin Biotech* 1990, 1, 166±171. J Natl Cancer Inst2000;92:179-181
25. https://www.rtog.org/ResearchAssociates/AdverseEventReporting.aspx
26. N. Ariyoshi, Y. Takahashi, M. Miyamoto, *et al*., Structural characterization of a new variant of the *CYP2A6* gene (*CYP2A6*1B*) apparently diagnosed as heterozygotes of *CYP2A6*1A* and *CYP2A6*4C*, Pharmacogenetics 10 (2000) 687–693.
27. L.W. Harries, M.J. Stubberns, D. Forman, G.C.W. Howard, C.R. Wolf, Identification of genetic polymorphisms at the glutathione-S-transferase P1 locus and association with susceptibility to bladder, testicular and prostate cancer, *Carcinogenesis* 18 (1997) 641-644.
28. W. Tan, G.F. Chen, D.Y. Xing, C.Y. Song, F.F. Kadlubar, D.X. Lin, Frequency of *CYP2A6* gene deletion and its relation to risk of lung and esophageal cancer in the Chinese population, *Int. J. Cancer* 95 (2001) 96–101.
29. Z. Topcu, I. Chiba, M. Fujieda, T. Shibata, N. Ariyoshi, H. Yamazaki, F. Sevgi- can, M. Muthumala, H. Kobayashi, T. Kamataki, *CYP2A6* gene deletion reduces oral cancer risk in betel quid chewers in Sri Lanka, *Carcinogenesis* 23 (2002) 595-598.
30. M. Nakajima, T. Fukami, H. Yamanaka, E. Higashi, H. Sakai, R. Yoshida, J.T. Kwon, H.L. McLeod, T. Yokoi,
31. Comprehensive evaluation of variability in nico- tine metabolism and *CYP2A6* polymorphic alleles in four ethnic populations, *Clin. Pharmacol. Ther*. 80 (2006) 282-297.
32. S.T. Saarikoski, A.V.M. Reinikainen, S. Anttila, A. Karjalainen, C. Malaveille, H. Vainio, K. Husgafvel-Pursiainen, A. Hirvonen, Combined effect of polymorphic GST genes on individual susceptibility to lung cancer, *Int. J. Cancer* 77 (1998) 516–521.
33. M. Miyamoto, Y. Umetsu, H. Dosaka-Akita, *et al*., *CYP2A6* gene deletion reduces susceptibility to lung cancer, *Biochem. Biophys. Res. Commun.* 261 (1999) 658-660.



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