Original Article

Treatment outcomes in poor metabolizers of cytochrome P450 2A6 with concurrent chemoradiotherapy in locally advanced head- and neck-squamous cell carcinoma

ABSTRACT

Introduction: Human Cytochrome 2A6 (CYP2A6) is involved in the oxidative metabolism of the nicotine to the inactive cotinine. CYP2A6 is a primary enzyme in nicotine metabolism, the enzyme has been proposed as a novel target for smoking cessation.

Materials and Methods: A total of 70 male patients of locally advanced head- and neck-squamous cell carcinoma confirmed by histopathological examination were enrolled in this study. All patients received concurrent chemoradiotherapy (total dose of 70 Gray in 35 fractions in 7 weeks with concurrent tablet capecitabine 1250 mg/m^2/day). Response assessment was based on response evaluation criteria in solid tumor criteria. Total ribonucleic acid (RNA) was isolated from the whole blood of all patients by TRI REAGENT BD (SIGMA USA) followed by real-time polymerase chain reaction assay which was done in studying messenger RNA (mRNA) expression of Excision Repair Cross Complementation Group 1 in blood lymphocytes of patient.

Results: The most common stage prevalent was Stage IV A in 28 (56%) patients followed by Stage III in 16 (32%) patients. Out of 70, 20 (28.6%) patients defaulted for treatment, so the analysis was done in 56 patients. A total of 19 (34%) patients had a complete response (CR) and 17 (30%) patients had no response. In all the patients who had CR, posttreatment relative quantification (RQ) expression levels were high. Among nonresponders only three had higher RQ folds and the rest 14 had lower RQ folds.

Conclusion: Posttreatment expression levels of CYP2A6 were found to be a better predictor for tumor response to the treatment than the pretreatment expression levels. Almost all the patients having higher RQ folds had CR and those having lower RQ folds had either no response or progressive disease on follow-up visits.

Keywords: Chemoradiotherapy, cytochrome P450 2A6, head- and neck-squamous cell carcinoma

INTRODUCTION

The head- and neck-squamous cell carcinomas (HNSCCs) (Sanderson) refer to a group of biologically similar cancers originating at different sites in the upper aerodigestive tract. Head- and neck-cancers are one of the most common cancers globally.[1] While in India, it accounts for one-fourth of male cancers and one-tenth of female cancers.[2]

Head- and neck-region consist of oral cavity, nasopharynx, oropharynx, hypopharynx, and larynx. Various risk factors are related to the development of head- and neck-cancer, these includes tobacco use, frequent and heavy consumption of alcohol, prolonged sun exposure, human papillomavirus (HPV)

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infection, Epstein-Barr virus infection, poor oral/dental hygiene, environmental/occupational inhalants, poor nutrition, and premalignant lesions such as submucosal fibrosis, leukoplakia, and erythroplakia.[3,4] Occupational exposures are associated with the development of sinonasal tract tumor.[5] Smoking is an independent risk factor in 80%–90% of patients.[6,7] Tobacco users have 5–25 folds increased risk of the oral cavity and oropharyngeal cancers.[8] The effect of alcohol and tobacco may be synergistic, most pronounced in pharyngeal and oral cavity cancer.[9] Head- and neck-cancer patients have an increased risk for developing a second primary tumor, this may be attributed to the field effect associated with tobacco and alcohol use.

Over the past two decades, HPV-positive squamous cell carcinoma has emerged as a distinct subset of HNSCC.[10] The patient with younger age group without a strong history of tobacco and ethanol use, and with history of multiple sex partners are the major population in this group. The most important prognostic factors are tumor size (T) and nodal (N) status. The probability of cervical lymph node involvement depending on the site and size of the lesion, T status, and depth of invasion.[11]

The identification of molecular markers that can predict the outcome of cancer is crucial in the management of patients with locally advanced head- and neck-cancers. Alkylating agent cisplatin is the main component of concurrent chemoradiotherapy (CCRT) which forms the deoxyribonucleic acid adducts, that cause inter and intrastrand cross-linking. The cytochromes P450 (CYPs) constitute the major enzyme family that catalyze the oxidative biotransformation of most drugs. Human CYP2A6 has been recognized as the major isoform involved in the oxidative metabolism of the psychoactive tobacco ingredient nicotine to the inactive cotinine. Because of its major contribution to nicotine metabolism, the enzyme has been proposed as a novel target for smoking cessation.[12] CYP2A6 also plays an important role as a modifier of smoking habits due to its participation in nicotine clearance.

The aim of this study was to study the expression of CYP 2A6 messenger ribonucleic acid (mRNA) in peripheral blood lymphocytes of head- and neck-cancer patients. Objectives were to study the effect of treatment in alterations of mRNA expression and to establish an association between mRNA expression and treatment response.

MATERIALS AND METHODS

The study was approved by the institutional ethical committee and patient consent was obtained for the study. Patients suffering from head- and neck-carcinoma, visiting the outpatient department of Radiotherapy from October 2018 to July 2019 were included in this study. The study group comprised 56 cases of locally advanced HNSCC confirmed by histopathological examination and were advised a combined modality treatment of chemoradiotherapy. All the cases included in the study belonged to the same ethnic group (Indo-European community) of North India.

Informed consent from all the patients was obtained before the inclusion in the study. All the patients were evaluated with the thorough history, physical examination, and routine blood investigations. They completed a questionnaire covering medical, residential, and occupational history. Information pertaining to dietary habits, family history of disease, smoking, tobacco chewing, and alcohol consumption were also obtained in the questionnaire filled by the patients. Those having a concurrent illness, defaults during the treatment, <6 months life expectancy, metastatic disease, and postoperative patients were excluded from the study.

All the patients were staged according to TNM classification (as per the AJCC 8th edition). Treatment consisted of CCRT using a total dose of 70 Gray (Gy) in 35 fractions given as 5 fractions per week in 7 weeks along with tablet capecitabine 1250 mg/m^2/day. Response was categorized as complete response (CR), partial response, or no response based on Response evaluation criteria in solid tumor assessment criteria. Toxicities were graded according to the Radiotherapy Oncology Group toxicity criteria.[13] Total RNA was isolated from the whole blood of the patient and control group by TRI REAGENT BD (SIGMA USA) for which the protocol has already been standardized in Indian Institute of Toxicology Research Laboratory. Real-time polymerase chain reaction (PCR) was used in studying the mRNA expression of Excision Repair Cross Complementation Group 1 in blood lymphocyte of the patient. The mRNA expression was studied before treatment, just after the completion of treatment, and monthly during follow-up to 6 months. The expression of mRNA was studied by PCR-restriction fragment length polymorphism technique.

OBSERVATION AND RESULTS

Out of 56 patients enrolled in the study, 30% of the patients belonged to the age between 51 and 60 years followed by 26% of the patient between 31 and 40 years of age [Table 1]. The mean age at presentation was 48 years with a standard deviation (SD) of 13.229 and 95% confidence interval (CI) (lower limit: 44.42; upper limit: 51.94). The mean age for controls was 45.54 years with SD of 13.008 [Table 2]. The oral cavity was the most common primary site involved, in 24 (52%) patients followed by

Table 1: Patient characteristic

Variable	Category	*n* (%)
Age (years) (median=28)	≤30	12 (24)
	31-40	25 (50)
	41-50	18 (36)
	51-60	28 (56)
	>60	17 (34)
Sex	Male	50 (100)
	Female	0
Comorbidity	Present	12 (24)
	Absent	38 (76)
Tobacco use	Present	44 (88)
	Absent	6 (12)
Alcohol use	Present	14 (28)
	Absent	36 (72)
Site	Oral Cavity	26 (52)
	Oropharynx	12 (24)
	Larynx	11 (22)
	Others	1 (2)
Stage-wise distribution (as per AJCC 8th)	I	0
	II	0
	III	16 (32)
	IVA	28 (56)
	IVB	6 (12)
	IVC	0
Histological differentiation	Well differentiated	15 (30)
	Moderately differentiated	22 (44)
	Poorly differentiated	3 (6)
	Undifferentiated	10 (20)
Response in RQ fold of mRNA	Complete response	19 (38)
	Partial response	8 (16)
	No response	17 (34)
	Progressive disease	6 (12)

n: Number of patients, AJCC: American Joint Cancer Committee, RQ: relative quantification, mRNA: messenger ribonucleic acid

12 (24%) patients of oropharynx [Table 1]. All the patients had squamous cell histology with variation in the grade. The most common histopathological grade found in our study was moderately differentiated (44% patients) followed by well differentiated (30% patients). The most common stage prevalent in our study was stage IV A in 56% of patients followed by stage III in 32% of patients.

In the study, 44 (88%) of patients consumed one or the other form of tobacco, the most common form of being tobacco chewing (66%) followed by smoking (62%). Smoking was graded according to "packs per year" smoked. Patients were found to smoke 1–90 pack years, with the majority falling in the range of ≤5 packs per-year. Median pack-years consumed was 4.50. It was also found that 26% of patients consumed tobacco both in the form of smoking and chewing whereas 26% of tobacco users also consumed alcohol. Among never drinkers, cigarette smoking was associated with an increased risk of head- and neck-cancer (odds ratio for ever versus never smoking = 2.13, 95% CI, 1.52–2.98), and there were clear dose-response relationships for the frequency, duration, and the number of pack-years of cigarette smoking. Approximately, 24% (95% CI, 16–31) of head- and neck-cancers among nondrinkers in this study would have been prevented if these individuals had not smoked cigarettes.

Table 2: Association of different demographic and clinical markers with treatment outcomes

Variables	Outcomes	
	Responders, *n* (%)	Nonresponders, *n* (%)
Age (years)		
≤50	14 (28)	12 (24)
>50	20 (40)	4 (8)
Primary site		
Oral cavity	17 (34)	9 (18)
Oropharynx	9 (18)	3 (6)
Larynx	7 (14)	4 (8)
Others	1 (2)	0
Stage (as per AJCC 8th edition)		
I	0	0
II	0	0
III	11 (22)	5 (10)
IVA	19 (38)	9 (18)
IVB	4 (8)	2 (4)
IVC	0	0
Histopathological differentiation		
WD, MD	24 (48)	13 (26)
PD, UD	10 (20)	3 (6)

n: Number of patients, AJCC: American Joint Cancer Committee, WD: Well differentiated, MD: Moderately differentiated, PD: Poorly differentiated, UD: Undifferentiated

In all the patients having CR, posttreatment expression levels were high whereas out of eight patients having PR, only two had higher relative quantification (RQ) fold posttreatment, and five patients had lower RQ fold. This suggests that, for CR, posttreatment RQ fold should be higher and vice versa.

Out of 17 patients who had no response, only three had higher RQ folds, and 14 patients had lower RQ folds further suggesting the significance of lower RQ folds in nonresponders. Out of the six patients who had progressive disease (PD), all had lower RQ folds posttreatment.

DISCUSSION

The head- and neck-cancer constitute 5.2%–6% of all cancers worldwide. According to the various studies, the prevalence of head- and neck-cancer with respect to total body malignancies varies from 10% to 40% in India. In India, head- and neck-cancer accounted for 30% of all cancers in males whereas in females 11%–16% of all sites of cancer.[14]

The treatment of HNSCCs depends on the stage, site of the primary tumor, and performance status of the patient. For early-stage disease, surgical resection with adequate margins with/without neck dissection is the treatment modality of choice. The decision for postoperative radiotherapy depends on the presence or absence of adverse features which include extracapsular nodal spread, positive margin, pathological T4 stage, N2 or N3 nodal disease, perineural invasion, and lymphovascular invasion.[15] Patients who are not the candidates for surgery (refuse surgery, comorbid illness), can be considered for definitive CCRT.[16] CCRT is the mainstay of the treatment for locally advanced inoperable head- and neck-cancers. The Meta-Analysis of Chemotherapy on Head- and Neck-Cancers demonstrated that the use of CCRT resulted in a 19% reduction in the risk of death and an overall 6.5% improvement in 5-year survival compared to the treatment with radiotherapy alone ($P < 0.0001$).[17] This benefit was attributable to a 13.5% improvement in locoregional control. There was also 2.9% (statistically significant) decrease in risk of distant metastasis.

The role of induction chemotherapy in locally advanced HNSCC is controversial. The Veterans Administration Cooperative Group and the European Organization for Research and Treatment of Cancer in a three-arm trial demonstrated that larynx preservation could be achieved without compromising overall the survival with induction chemotherapy followed by standard fraction (versus laryngectomy and postoperative radiotherapy) in advanced carcinoma of larynx and hypopharynx.[18] Chemotherapy given with radiotherapy serves as a radiosensitizer which enhances the activity of radiation, and imparts a direct cytotoxic effect to local tumor cells, and subclinical distant metastasis or spreading of disease beyond the radiation area. Despite an improved survival rate with CCRT against the radiation therapy alone, the prognosis in this group has, however, remained unchanged over the past many years. Even the most effective treatment regimens result in local control rates of 50%–70% and disease-free survival (DFS) of 30%–40%.[19] Thus, since the rate of recurrence is relatively high and the incidence of side effects is quite frequent, there is still a great need for improvement in treatment strategies.

Many biological factors that help regulate cell cycle control, apoptosis, angiogenesis, or invasive or metastatic potential have been proposed as prognostic determinants of HNSCCs. Examples include smoking, tobacco chewing, alcoholism, epidermal growth factor receptor expression, vascular endothelial growth factor receptor expression, tumor perfusion, and hypoxia. With the advances in treatment strategies for HNSCCs, newer targeted therapies are being added to the progress already achieved in the multimodality management of patients, although the problems of differences in drug response and adverse drug reactions are still of a grave concern. Cancer pharmacogenomics has fast emerged as a new and promising field for the early identification of genetic markers that can predict drug response or toxicity. This could greatly help in identifying genetic markers useful for the selection of optimal drugs, dose, and treatment duration on an individual basis resulting in improved drug efficacy and decreased toxicity.

This review focuses on the description of the contribution of genetic variations to chemotherapeutic toxicity and response in HNSCC patients. Hence, we were encouraged to study the expression of CYP 2A6 in HNSCC patients, as their expression is associated with nicotine metabolism and hence, nicotine (tobacco) dependence; which has a strong causal association with head- and neck-cancers.

In the present study, pretreatment expression levels of CYP 2A6 were markedly raised in cases as compared to controls. However, pretreatment levels were not found to be a better predictor of response than posttreatment expression levels. On univariate logistic regression analysis, patients who had a significant increase in CYP 2A6 expression level after treatment had 5.4 times higher chances of good response to treatment. However, on multivariate logistic regression analysis increased expression of CYP 2A6 after treatment failed to show any significant correlation with response. A study evaluated the role of CYP2A6 in modulating the treatment outcome in HNSCC cases.[20] The treatment response was poor, particularly in cases with at least 1 deletion allele of CYP2A6. The CYP2A6 genotype is correlated with the treatment efficacy of tegafur-based chemotherapy in metastatic gastric carcinoma patients. Patients with a wild/wild or wild/variant genotype were more likely than those with variant/variant genotype to have a good treatment outcome.[21]

Almost all the patients having higher RQ folds had CR in our study and those having lower RQ folds had either no response or PD on follow-up visits. Our study also suggests that treatment modifies CYP2A6 mRNA expression levels in comparison with pretreatment levels. In all the patients who have CR, posttreatment expression levels are high. Out of eight patients who had PR only two of them had higher RQ fold posttreatment and five patients had lower RQ fold suggesting further that for CR posttreatment RQ fold should be higher or vice versa.

Our study suggests that there is a correlation between treatment response and CYP2A6 mRNA expression level, however, it fails to explain the association with DFS and long-term overall survival,

due to short follow-up period. To reach a definite conclusion, longer follow-up periods with a large sample size are required to know the actual impact of CYP 2A6 overexpression on the long-term overall and disease-free survival.

CONCLUSION

There is a significant difference in the blood lymphocyte CYP 2A6 expression in the cases. Posttreatment expression levels are found to be a better predictor for tumor response to treatment than pretreatment levels. There is a scarcity of evidence supporting these findings. In view of the above, it is suggested that this study should be undertaken on a larger patient population with more patients included for follow-up to substantiate the findings of the study, before it could propose to be included in the routine clinical practice.

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Conflicts of interest

There are no conflicts of interest.

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