Impact of Molecular Predictors on the Response Rates in Head and Neck Cancer Patients – an Observational Study

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Abstract Squamous cell carcinoma of head and neck region account for more than 25 % of male and more than 10 % of female cancers in India (1). Head and neck cancer treatment includes a multidisciplinary approach involving all specialties. Concurrent chemo-radiation is the standard of care in most of the subsites (2). In spite of the multi-disciplinary approach, a plateau has been reached in terms of results with 5 year survival of locally advanced disease of around 30 % (3). In order to improve outcomes, there has been considerable interest in molecular profiling of head and neck cancers 4–10. However there is still significant paucity in terms of Indian data, hence the need for the study. The objectives are to assess the HPV-p16, EGFR and p53 status, to correlate HPV-p16, EGFR and p53 status with the response rates, to correlate HPV-p16, EGFR and p53 status with other factors like age, sex, tobacco use. Twenty five consecutive cases of histopathologically proven head and neck cancers were accrued. All patients were treated with external radiation to a dose of 66Gy in 33 fractions along with concurrent weekly cisplatin chemotherapy at a dose of 40mg/sqm. HPV-p16, EGFR and p53 mutation analysis was done on paraffin embedded histopathological blocks. PCR technique used for HPV-p16, EGFR and p53 status

detection. Response assessment was done based on RECIST criteria. Correlation of HPV, EGFR and p53 status on response was done. The EGFR positivity rate was 84 %, the p53 positivity rate was 76 % and the HPV p-16 positivity rate was 28 %. Out of 25 patients, 13(52%) had complete response, 7(28 %) had partial response, 3(12 %) had stable disease and 2(8 %) had progressive disease. On correlation of molecular profile with response, there was no statistical significance between EGFR status and response (p 0.5) or HPV-p16 and response (p 0.8). However, p53 positivity was approaching significance with respect to good response (p 0.07).

Keywords Head and neck cancer · Radiotherapy · Molecular predictors · EGFR · p16 · p53

Introduction

Squamous cell carcinoma of head and neck region account for more than 25 % of male and more than 10 % of female cancers in India. It has been postulated that there would be a substantial increase in the incidence of head and neck cancers in the coming decade. Estimated number of new cases by the year 2020 would be 1, 53,636 and 64,785 in males and females respectively [1].

Head and neck cancer treatment includes a multidisciplinary approach involving all specialties, concurrent chemo-radiation is the standard of care in most of the subsites of head and neck cancer [2]. In spite of the multi-disciplinary approach, a plateau has been reached in terms of results. It poses great challenges due to the aggressive behavior and high rates of local, regional as well as systemic failures. The results are

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quite dismal even in the present day of technological advancements with overall survival rates of 20–30 % at 5 years for advanced stage diseases.

As a basis of any successful treatment one needs to understand the pathogenesis of a disease, in order to maximize the potential areas of intervention so results can be optimised.

In this regard molecular predictors are seen as a way of understanding the pathogenesis and in-turn tailoring treatment approaches to target the various predictors.

As per the western literature HPV has shown to be associated in >30 % of oropharyngeal cancers. HPV is said to be positive in younger, non-smokers and usually associated with a better prognosis [3].

EGFR mutation data on head and neck cancers are proven to be of much value due to the availability of EGFR inhibitors but the data on its positivity in head and neck cancers is in its infancy. EGFR mutation is an indicator of aggressive nature of the disease and also an indicator of metastatic potential [4].

Increased p53 mutation rate is associated with tobacco and alcohol use in HNSCC and also with increased risk of progression to cancer [5].

Due to the aforementioned reasons, there has been considerable interest in molecular profiling of head and neck cancers. However there is still significant paucity in terms of Indian data, hence the need for the study.

Objectives

- To assess the HPV – p16, EGFR and p53 status
- To correlate HPV-p16, EGFR and p53 status with the response rates
- To correlate HPV-p16, EGFR and p53 status with other factors like age, sex, tobacco use.

Methods and Materials

Study Design

Prospective cohort study.

Sample Size 25 consecutive cases of histopathologically proven head and neck cancers were accrued.

Inclusion Criteria

- Histopathologically proven cases of head and neck cancer
- Treatment with radical intent

Exclusion Criteria

Post-op cases.

Methods

The molecular profiling was done on histopathological blocks using BioGenix kits (San Ramon, California, U.S.A).

HPV Testing Histopathological blocks were subjected to Immunohistochemistry to know the p16 status. p16 overexpression is an indicator of oncogenic transformation of persistent High risk HPV infection.

EGFR Testing EGFR testing done on paraffin embedded histopathological blocks using PCR technique.

p53 Testing p53 testing also done on paraffin embedded histopathological blocks. Immunohistochemistry technique used for p53 analysis.

Molecular Testing Protocol

p16, p53, EGFR

Slides are de-paraffinised and later hydrated using Xylene and Alcohol.

After 25 min retrieval of antigen the slides were exposed to peroxide block for 10 mins followed by power block for 10 min.

The slides were incubated with primary antibody (for HPV 16/P53/EGFR) for 1 h followed by buffer wash and later super enhancer and polymer HRP (for HPV 16/P53/EGFR) was added for 20 mins and 30 mins respectively.

Freshly prepared 1 drop of liquid DAB chromogen in 1 ml of stable DAB buffer was added to the tissue and incubated for 5 min at room temperature and washed with running water. Later it was counter stained with hematoxylin. Clearing and mounting was done using alcohol, xylene and DPX.

Interpretation The slides are screened for two things:

- 1 Intensity of staining scored from 0 to 3
- 2 Percent of cells which have taken the stain

Treatment Given

All patients were treated with external radiation to a dose of 66Gy in 33 fractions along with concurrent weekly cisplatin chemotherapy at a dose of 40 mg/sqm.

Table 1 Site wise distribution

Site	Number	Percent
Oral cavity	3	12
Oro-pharynx	16	64
Hypo-pharynx	4	16
Larynx	2	8

Response Assessment

- Response assessment based on RECIST Criteria.
- Correlation of HPV-p16 ,EGFR and p53 status on Response.

Statistics

All statistical analysis done on IBM SPSS v 20.0 software. Mean and standard deviation applied for nominal variables. Pearson's Chi Square test applied for analysis of variance.

End Points

Primary Incidence of HPV-p16 ,EGFR and p53 positivity in our patients and its correlation to Response Rates.

Secondary Correlation of other variables like Age, Sex, Tobacco, Socioeconomic Status on the HPV-p16 ,EGFR and p53 status.

Results

Demographics

- Mean Age of the study population: 59 years
- Sex: 76 % Male and 24 % Female
- Stage: I-8 % ,II-4 % ,III-36 % ,IV-52 %
- Tobacco Use: 92 % were tobacco consumers either in the smoke or smokeless form and 8 % were non-tobacco users
- Socio-Economic Class-76 % belonged to the low socio-economic class, 20 % to the middle socio-economic class and 4 % to the high socio-economic class (Table 1)

Results of Mutation Analysis (Fig. 1)

- The EGFR positivity rate was 84 %
- The p53 positivity rate was 76 %
- The HPV p-16 positivity rate was 28 %

Response Rates (Fig. 2)

Out of 25 patients, 13(52 %) had Complete Response, 7(28 %) had Partial Response, 3(12 %) had Stable Disease and 2(8 %) had Progressive Disease.

Fig. 1 Molecular profile

Fig. 2 Response to concurrent chemoradiation

Correlation of Molecular Profile with Response

- On correlation of molecular profile with response, there was no statistical significance between EGFR status and response (p 0.5) or HPV-p16 and response (p 0.8). However, p53 positivity was approaching significance with respect to response (0.073).
- Among other factors there was significant correlation between smoking and HPV-p-16 status (p 0.01). However there was no significant correlation between other factors like age, sex and socio-economic status (Table 2).

Discussion

Head and neck cancers are a heterogeneous group of malignancies in terms of etiology, biological behavior and prognosis. Although many clinic-pathological parameters have been

indicated to predict prognosis, no such markers are used in clinical practice today, apart from TNM staging. However, recent insights in molecular changes underlying HNSCC development have identified new potential prognostic indicators, such as HPV, EGFR and p53 status. In this regard various authors have assessed the impact of these molecular markers on various treatment related parameters.

HPV p16 and Outcome (Table 3)

In our study there was no statistically significant impact of p16 status on the response rates. There have been contrasting results with respect to p16 status and treatment outcomes across different sub-sites of head and neck squamous cell carcinoma, as in a study by Bova RJ et al. [6], the loss of p16 expression were associated with poor outcomes in carcinoma of the anterior tongue in terms of disease free survival and overall survival.

Contrastingly in a clinicopathological study by Yuen PW et al. [7], they found that downregulation of p16 contributed to cellular proliferation, resulting locally in a more advanced tumour. However, p16 expression had no prognostic significance for nodal metastasis or survival.

In a study by Reimers et al. [8], there was significant impact of p16 positivity. Patients with p16-negative OSCC had a 4-fold increase in their risk of death from any cause, and a 7.5-fold increased risk of recurring cancer, compared with patients with p16-positive tumors. Despite the increased outcome of p16-positive and HPV positive tumors, they were more likely to be poorly differentiated.

Table 2 Correlation with response

Response	p53		EGFR		HPVp16	
	-	+	-	+	-	+
CR	1	12	2	11	10	3
PR	2	5	1	6	5	2
SD	1	2	0	3	2	1
PD	2	0	1	1	1	1
pValue	0.073		0.5		0.8	

Table 3 HPV p16 and prognosis

Study	Number	Year	Outcome
Bova RJ et al	148	1999	loss of p16 expression was associated with poor outcomes in carcinoma of the anterior tongue in terms of DFS and OS.
Yuen PW et al.	225	2002	p16 associated with locally more advanced tumor but no significance for nodal mets or survival
Reimers N et al	106	2007	p16-negative OSCC had a 4-fold increase in their risk of death, and a 7.5-fold increased risk of recurring cancer.
Present	25	2013–14	No statistically significant impact on the response

EGFR and Outcome (Table 4)

In our study the impact of EGFR on response rates was not statistically significant, however this is not the case across other trials. EGFR over-expression has been consistently associated with poor prognosis in head and neck cancer.

In a study by Reimers N et al. [8], EGFR expression did not reach significant levels in either univariate or multivariate analysis. The EGFR-positive cases tended to have worse 5-year disease-free survival and worse 5-year overall survival rates compared with EGFR-negative cases.

Ang KK et al. [9] analysed the impact of EGFR status on the survival and pattern of relapse in head and neck cancer. They found that, The OS and DFS rates of patients with high EGFR-expressing HNSCCs were highly significantly lower (P0.0006 and P0.0016, respectively) and the LR relapse rate was highly significantly higher (P0.0031) compared with those of patients with low EGFR-expressing HNSCCs. However, there was no difference in the distant metastasis rate between the two groups.

P53 and Outcome (Table 5)

In our study there was no statistical significance in response rates in p53 mutation positive tumors (p 0.073). However p53 positive tumors were associated with higher percentage of complete response 12 of the 13 tumors achieving complete response were p53 positive. Although there is some

controversy regarding the impact of p53 mutations on treatment outcome, across various trials the general consensus is that p53 mutation is associated with poor outcome as in a study by Geisler et al. [10], patients whose tumors showed increased p53 protein expression had over twice the risk of all-cause mortality after 550 days [hazard ratio (HR), 2.7; 95 % confidence interval (CI), 1.07–6.66] and three times the risk of dying from cancer-specific causes after 550 days.

In contrast in a study by Pruneri et al. [11], they found no prognostic significance for p53 on overall survival.

The long term results of our study would probably give a better idea as to the actual impact of these molecular markers on the prognosis in head and neck cancer.

Strengths

Analysis of three independent prognostic molecular variables namely, HPV-p16, EGFR and p53 status.

Clinical correlation in-terms of response assessment.

Weakness

A very small sample size, which means the power of the study is inadequate to come to a definitive conclusion.

Conclusion

The HPV, EGFR and p53 positivity rates were in concordance with hitherto published literature. The HPV

Table 4 EGFR and outcome

Study	Number	Year	Outcome
Ang KK et al	155	2002	The OS and DFS rates of patients with high EGFR-expressing HNSCCs were highly significantly lower (P0.0006 and P0.0016, respectively) and the LR relapse rate was highly significantly higher (P0.0031)
Reimers N et al	106	2007	EGFR-positive cases tended to have worse 5-year DFS and worse 5-year OS rates
Present	25	2013–14	No statistically significant impact on the response

Table 5 P53 and outcome

Study	Number	Year	Outcome
Pruneri et al	149	1998	no prognostic significance of p53 on overall survival.
Greisler et al	171	2002	p53 positive cases had over twice the risk of all-cause mortality and three times the risk of dying from cancer-specific causes after 550 days.
Present	25	2013–14	Approaching significant impact on the complete response rates (p 0.073)

and EGFR did not have significant impact on response rate however p53 positivity was associated with higher percentage of complete response rate which was approaching statistical significance.

Future Perspective

As a future perspective, the same patients would be subjected for a long term follow up and analysed for survival statistics. It is to be seen whether HPV-p16, p53 and EGFR status would have any impact on long term outcomes. In terms of tailoring treatment, it might be possible to improve therapeutic outcome in tumors with inactive p16 by restoration of p16 activity, p16 adenovirus mediated gene therapy is also being tried. Targeted therapy against EGFR mutations is already in vogue and similar trials for p53 mutations are expected in the near future.

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Compliance with Ethical Standards

Conflict of Interest None

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