Medical and Research Publications
International Open Access

Research Article **Journal of MAR Oncology (Volume 3 Issue 6)**

Study the Pattern of Ki-67, EGFR, p53 and HPV-DNA in Squamous Cell Carcinoma Head and Neck

Dr. Antara Sanyal [1]*, Dr. Vipul Nautiyal [1], Dr. Meenu Gupta [1], Dr. Nadia Shirazi [2], Dr. Mushtaq Ahmad [1], Dr. Sunil Saini [3].

1.Department of Radiation Oncology, Cancer Research Institute, Himalayan Institute of Medical Sciences, Swami Rama Himalayan University, Dehradun, Uttarakhand, India.

2. Department of Pathology, Himalayan Institute of Medical Sciences, Swami Rama Himalayan University, Dehradun, Uttarakhand, India.

3. Department of Surgical Oncology, Cancer Research Institute, Himalayan Institute of Medical Sciences, Swami Rama Himalayan University, Dehradun, Uttarakhand, India.

Corresponding Author: Dr. Antara Sanyal, M.D. Radiation Oncology, Uttarakhand, India. https://orcid.org/0000-0001-8378-5672

Received Date: April 25, 2022

Published Date: May 01, 2022

Abstract

Objectives:

To study the pattern of Ki-67, EGFR, p53 and HPV-DNA in Squamous Cell Carcinoma Head and Neck.

Methods:

Prospective data collection of HNSCC patients from January 2019 to January 2020 who were treated at our centre.

Inclusion Criteria: *Patients with Squamous Cell Carcinoma of the Head and Neck.*

Exclusion Criteria: *Any previous oncological treatment except Biopsy/FNAC; Any other histology in Head and Neck.*

Histopathology and IHC was performed for molecular characterization of tumours. DNA-PCR was performed for HPV-DNA in oral swabs. The patients were managed as per institutional protocol and standard guidelines depending upon the site of Primary tumour and stage.

Results:

Total patients (N)=51; Age Group (Years) <50: 31.4%; 48 male (94.1%); T-Stage: T4: 35.2%; N-Stage: N3: 13.7%; Site-Wise (predominantly): Oral Cavity: 23.5%, Oropharynx: 45%; Risk Categorization: High Risk (EGFR 2+ & 3+ / p53 2+ & 3+/ Ki-67: > 25%): 44 (86.3%) and Low Risk (EGFR / p53 / Ki-67 1+/Negative): 7 (13.7%). On Kaplan-Meier Survival Analysis,it was observed that survival probability of low risk was better than high risk.

Conclusions:

A battery of biomarkers in addition to histopathology is an unmet need for predictive outcomes with certain treatment modalities and prognostication of the primary disease in Head and neck and should be incorporated in management guidelines.

Keywords: *HNSCC; NACT; PORT; IHC; EGFR; p53; Ki-67; HPV-DNA; Oral Cavity, Oropharynx.*

Introduction

Being the 6th most common cancer worldwide (1), in Indian Scenario, HNSCC is predominantly diagnosed in men and ranked 5th most common malignancy amongst women (2). The Field Cancerization theory helped establish emergence of malignancy in the head and neck region due to various genetic alterations (3) including TSGs (tumour suppressor genes) amongst others (4). Earlier, management of head and neck malignancies was initially limited to conventional prognostic factors commonly practiced clinically however in most of these cases, inadequacy of these factors and inability to differentiate these tumors on the basis of response to treatment and clinical outcome despite being of the same clinical stage was a major confounding factor. Henceforth, the ground for incorporation of molecular and biological prognostic markers came into play to better reflect the biological diversity that a disease may encompass and to tailor treatment plans according to it and the clinical stage (5). Varied alterations in oncogenes and receptors for growth drivers such as and not limited to p53 and EGFR are attributed for molecular carcinogenesis of HNSCC. Thus, identification of individual disease risk and prognosis with the study of molecular biomarkers has become significant (6). In this study, we observed the pattern of Ki-67, p53, EGFR after risk categorization in HNSCC along with its correlation with clinico-pathological parameters and its effect on treatment outcomes.

Materials and Methods:

Patient Selection

This observational cross-sectional study was conducted in the Department of Radiation Oncology, HIMS - 51 patients of newly diagnosed Squamous Cell Carcinoma Head and Neck were included after written consent. Exclusion Criteria: Any previous oncological treatment excluding Biopsy/FNAC, any other histology.

All relevant data was collected in the form of pre-developed data reporting forms, labs and radiological investigations. Staging was done as per AJCC 2017 Edition.

Immunohistochemistry, Interpretation and Scoring

H & E staining of paraffin blocks followed by IHC for Ki-67, EGFR and p53 was performed as per protocol in the Department of Pathology (BIOGENEX Company Kit). The expression of Ki-67, EGFR and p53 were scored semi-quantitatively: P53: Mouse Monoclonal, Clone- D07, Conc. IVD, Cell Marque, 453R-94; EGFR: Rabbit Monoclonal, Clone- SP84, Conc. IVD, Cell Marque, 414R-14; Ki-67: Rabbit Monoclonal, Clone-30-9, RTU. IVD, VENTANA, 790 - 4286 -5M.

(i) EGFR Immunostain Scoring (membranous staining of tumour cells): '0': No Staining in <10%, '1+': Incomplete staining in >10%, '2+': Weak-moderate complete staining in >10%, '3+': Strong and complete staining in >10%.

(ii) Scoring of Ki-67 and p53 (Positivity of Nuclei [%]): '0': Complete lack of positivity, '1+': 5-10, '2+': 10-30, '3+': 30-50, '4+': >50.

(iii) DNA-PCR for Human Papilloma Virus: HPV DNA was analyzed by PCR (Sample Type: Oral Swab; HPV-DNA including oncogenic and non-oncogenic types - "16, 18, 31 , 33 , 35 , 39 , 45 , 51 , 52 , 56 , 58 , 59 , 66 and 68").

Risk Stratification: We have grouped IHC Markers as-

High Risk: Ki-67: >25%, p53: 2+/3+, EGFR: 2+/3+

Low Risk: Ki-67: ≤ 25%, p53: 1+/Negative, EGFR: 1+

Treatment Modalities:

Depending on the site of Primary and stage, in cases of locally advanced disease, patients underwent 2-3 cycles of Induction chemotherapy followed by depending upon the site-specific response, either were subjected to Surgery + PORT or Definitive CRT or Palliative EBRT.

Statistical Analysis:

A database was constituted using electronic spreadsheets (MS Excel), freely available software solutions (SPSS Version 20), NTCP software to store and manage the collected data. Data interpretation along with the analysis of obtained results was carried out using the following tests: Qualitative Data was expressed in terms of frequency/percentage; Quantitative Data were expressed in terms of mean ± SD; Paired t - test was applied for the distribution of difference between two groups was normally distributed; a p-value less than 0.05 was considered statistically significant.

Results

Baseline characteristics have been summarized in Table 1.

Total patients (N)=51; 48 patients were male (94.1%) with 3 female patients (5.9%); with majority of the patients in the age Group of <50 years: 16 patients (31.4%); T-Stage: T4: 18 patients (35.2%), with most common N-stage being N2: 17 patients (33.3%), most common site of involvement: Oropharynx: 45% with Stage-IV: 31 patients (60.78%). Risk Stratification: High Risk: 44 (86.3%) and Low Risk: 7 (13.7%).

Table 1. Baseline Characteristics

Parameters		Total (N)=51; Frequency (n) (%)
Age Groups (years) -	< 50	16 (31.4)
	50-60	15 (29.4)
	61-70	13 (25.5)
	>70	7 (13.7)
Sex -	Male	48 (94.1)
	Female	3 (5.9)
Tobacco -	Users	44 (84.6)
	Non-Users	7 (13.5)
T-Stage -	T4	18 (35.2)
	T3	15 (29.4)
	T2	14 (27.4)
	T1	4 (7.84)
N-Stage -	N3	7 (13.7)
	N2	17 (33.3)
	N1	11 (21.5)
	N0	16 (31.3)
Overall Stage -	I	2 (3.7)
	II	5 (9.8)
	III	13 (25.4)
	IV	31 (60.7)
Site-Wise Distribution-	Oral Cavity	12 (23.5)
	Oropharynx	23 (45.0)
	Larynx	14 (27.4)
	Hypopharynx	2 (3.9)

Correlation of Risk Categories with Clinicopathological Parameters

Of the 44 patients in the high risk category with overexpression of EGFR and p53 and a high proliferative index- it was observed in 37/51 (84.1%) were tobacco users vs. 7/51 (15.9%) non-users; 38/51 patients (86.4%) had a MDSCC grade of tumour with 6 patients (13.6%) harbouring a poorly differentiated tumour (p-value 0.427); majority of stage-IV cases had a high proliferative index (27/31), over-expression of EGFR (21/30) and p53 (26/31) with all of them being HPV-negative tumours (p-value 0.912).

Correlation of Risk Categories with Treatment Responders and Non-Responders

37 patients received CTRT, of these, 27 patients received induction chemotherapy and it was observed that only 11 patients responded (partial response/complete response) post-induction chemotherapy of which majority had a high proliferative index, EGFR and p53 over-expression (10/11) and HPV negative tumours however majority of patients (33/37) had complete response post-definitive CRT despite a similar molecular profile- 27 (87.1%) in high risk and 6 (100%) in low-risk category.

Survival Analysis

Between Treatment Arms: In terms of mean survival time, the average survival time is higher (i.e. 9.6 months) in IC + Surgery + CTRT as compared to Surgery + CTRT (i.e., 5.75) (p-value 0.087).

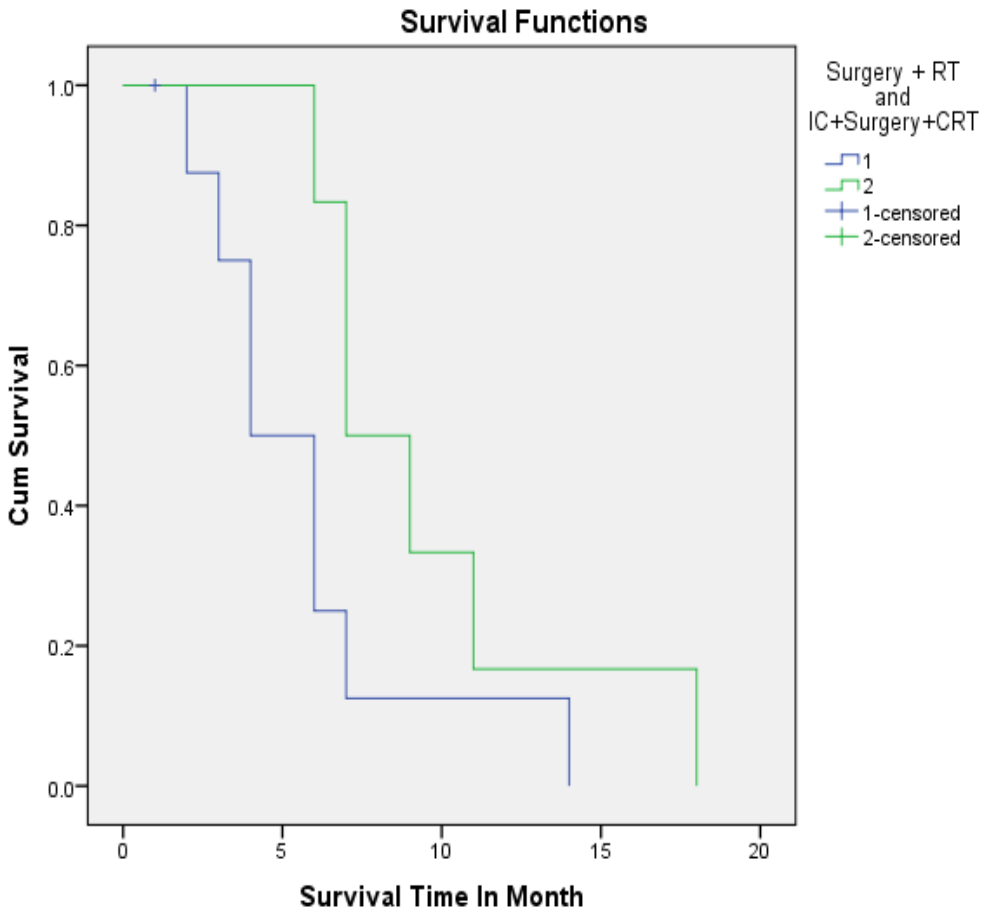

Figure 1

Between High-risk and Low-risk categories: it was observed to be 7.0 months in high-risk category and 10.0 months in low-risk category- with average survival time being higher in low-risk category (p-value 0.178).

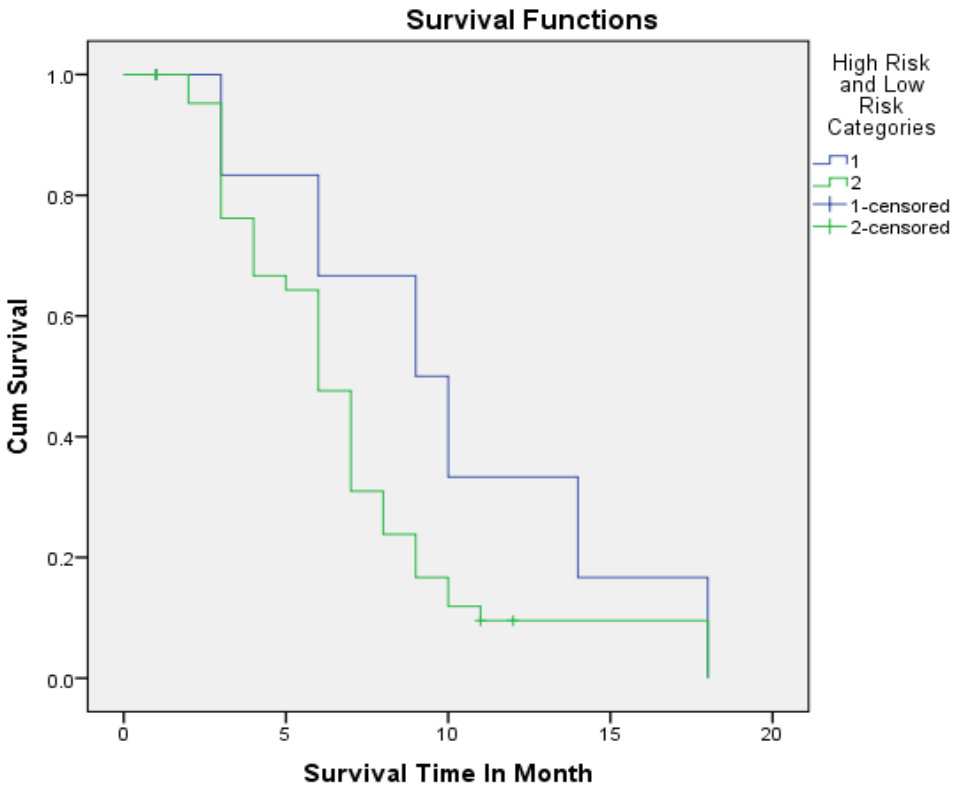

Figure 02

Discussion

HNSCC being the 6th most commonplace malignancy; accounting for ~50-70% of total cause mortality and highest incidence in a country like ours (7), has been a widely researched in the last few decades in terms of etiology, pathogenesis, diagnostic evaluation and approach to treatment modalities. Hence for a more precise and accurate reflection of the tumour biology and aggressiveness, testing for a series of markers at the same time is required to find out the ones of paramount importance for prognostication and tailoring treatment accordingly for a comparable response to treatment with reduction in treatment-related toxicities (5). In our study, we were able to confirm male predominance (94.1%) in terms of HNSCC development with consistent history of alcohol and tobacco abuse whereas only 5.9% affected patients were female (1). In this study, there were 84.6 % (44/51) cases who were tobacco users whereas 13.5 % (7/51) of the cases had no history of tobacco usage in any form and is concordance to previous studies (7). In concordance to previous study on HNSCC for most common site of involvement (8), similar to that, our study also observed most common site of involvement in oropharynx (23/51) (45.09%) followed by larynx (14/51) (27.45%) and others.

As documented in previous studies, our study also showed that p53 mutated status significantly correlated with tobacco usage (90.2%) and with EGFR over-expression (88.2%). However, there were 13.72% users who had a p53-negative HNSCC. A possible reason for p53 negative status in tumour tissue is the limitation of the sensitivity of p53 IHC for detecting the p53 mutation as previously. Nylander et al. also reported that HNSCCs with large p53 mutations, such as deletions causing frame shifts or stop codons, which are reported to cover 12% of OPSCC cases with p53 mutations, produced tumors that were negative for p53 by IHC (9). In the present study, the patients who were p53-positive showed poorer prognosis than those who were p53 negative. In terms of response to treatment, of the 27 patients who had received induction chemotherapy as per our institutional protocol for LAHNC (locally advanced head and neck cancer), it was observed that: 11 patients (40.74%) had responded despite 10 of them harboring a high proliferative index (Ki-67 > 25%), 8 patients with EGFR over-expression, 9 of them with p53 mutation and 11/11 negative for HPV-DNA PCR. It was also observed that 16/27 (59.25%) were non-responders with EGFR overexpression with a majority with a low Ki-67 but p53 mutation present. This is in concordance to a study by Hitt et al. documenting poor response to induction chemotherapy (10). In terms of response to Definitive CTRT (37 patients), 33 had complete response in patients with EGFR over-expression with combined Ki-67/p53 mutated and is in concordance to a study by Kumar et al. (11); however 4 patients had a residual disease and 26 had recurrence post-treatment completion. As HPV positive patients were very scarce in our study with only 2 patients and is in concordance to a study by Rajesh D. et al (12).

The correlation and prognostication based on IHC markers and HPV-DNA positive/negative status is a burden in a set-up where logistic issues and poor compliance and default to treatment is a common confounding factor leading to poor accrual and statistically insignificant results despite documented evidence of response based on IHC marker status.Molecular subtyping is already common in some malignancies such as breast cancers, and shows great potential for furthering our understanding of OPSCCs. This report may help to generate study models with larger cohorts for improving treatment based on the classification of different molecular subtypes.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest

References

1. Ferlay J, Soerjomataram I, Ervik M, Dikshit R, Eser S, Mathers C, et al. Globocan 2012. Estimated cancer incidence, mortality and prevalence worldwide in 2012 v1.0. International Agency for Research on Cancer. https://publications.iarc.fr/Databases/Iarc-Cancerbases/GLOBOCAN-2012-Estimated-Cancer-Incidence-Mortality-And-Prevalence-Worldwide-In-2012-V1.0-2012/ Accessed July 31 2018.

2. Sankaranarayanan R, Swaminathan R, Lucas E. Cancer survival in Africa, Asia, the Caribbean and Central America (SurvCan). International Agency for Research on Cancer. https://survcan.iarc. fr/ survivalcitation.php/ Accessed July 31 2018.

3. Kumar A, Sharma S, Ahlawat B, Sharma A, Prasad S, Chaudhary N. Synchronous second primary upper aerodigestive tract tumour emphasizing field cancerization: a case report. J Med Res Prac. 2015;4:16-20.

4. Howard JD, Lu B, Chung CH. Therapeutic targets in head and neck squamous cell carcinoma: identification, evaluation, and clinical translation. Oral Oncol. 2012;48(1):10-17.

5. Shah NG, Trivedi TI, Tankshali RA, Goswami JV, Jetly DH, Shukla SN, et al. Prognostic significance of molecular markers in oral squamous cell carcinoma: A multivariate analysis. Head & Neck. 2009;31(12):1544-56.

6. Hashmi AA, Hussain ZF, Aijaz S, Irfan M, Khan EY, Naz S, et al. Immunohistochemical expression of epidermal growth factor receptor (EGFR) in South Asian head and neck squamous cell carcinoma: association with various risk factors and clinic-pathologic and prognostic parameters. World J Surg Onc. 2018; 16:1-9.

7. Bukhari MH, Saba K, Qamar S, Khalil E, Niazi S. Histopathological and Immunohistochemistry Observations Based Bukhari Grading for Squamous cell carcinoma. Acta Medica International. 2015;2(2):54-60.

8. Sharma JD, Baishya N, Kataki AC, Kalita CR, Das AK, Rahman T. Head and neck squamous cell carcinoma in young adults: A hospital-based study. Indian J Med Paediatr Oncol. 2019;40(S1)18-22.

9. Nylander K, Dabelsteen E, Hall PA. The p53 molecule and its prognostic role in squamous cell carcinomas of the head and neck. J Oral Pathol Med. 2000;29(9):413-25.

10. Hitt R, Ciruelos E, Amador ML, Benito A, Sanchez JJ, Ballestin C, Cortes-Funes Phase III study comparing cisplatin plus fluorouracil to paclitaxel, cisplatin, and fluorouracil induction chemotherapy followed by chemoradiotherapy in locally advanced head and neck cancer. J Clin Oncol. 2005;23(34):8636-45.

11. Kumar B, Cordell KG, Lee JS, Worden FP, Prince ME, Tran HH, et al. EGFR, p16, HPV Titer, Bcl-xL and p53, sex, and smoking as indicators of response to therapy and survival in oropharyngeal cancer. J Clin Oncol. 2008;26(19):3128-37.

12. Rajesh D, Mohiyuddin S M, Kutty A V, Balakrishna S. Prevalence of human papillomavirus in oral squamous cell carcinoma: A rural teaching hospital-based cross-sectional study. Indian J Cancer. 2017;54:498-501.