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ORIGINAL ARTICLE

Molecular Marker as a Prognostic Indicator in Head and Neck Cancer Patients

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Abstract

Head and neck cancer forms the major burden of cancer in the developing countries. Despite advancement in the treatment approach of head and neck cancer in terms of surgery, chemotherapy and radiotherapy overall long-term survival remains low due to uncontrollable persistent and recurrent disease. This low survival rate has demanded for the need for newer treatment approaches and prognostic markers. In a previously published study "impact of molecular predictors on the response rates in head and neck Cancer patients" by Koushik et al. assessed the impact of molecular markers like HPV, P53, and EGFR status along with other prognostic factors like tobacco use, age, sex, and socio-economic status on response to treatment of head and neck cancer patients. Our present study is intent to provide update of the impact of those molecular markers on survival. Objective of our study is to correlate the HPV, EGFR, and P53 status with the survival rate of the head and neck cancer patients. Twenty-five histologically proven head and neck cancer patients were assessed for HPV, EGFR, and P53 status who underwent chemoradiation to a dose of 66 Gy in 33 fraction along with weekly cisplatin of 40 mg/m^2, and all treated patients were followed up to a minimum of 3 years and analyzed for the survival. We found that 3-year survival for complete responders after treatment is 61.5% and partial responders, 57.1%; stable disease is 33.3%, and progressive disease is 0%. A 3-year survival for HPV-positive patients is 57.4% ($p = 0.973$), EGFR-mutated patients is 47.62% ($p = 0.593$), and P53-mutated patients is 57.89% ($p = 0.378$).

Keywords Molecular marker · Head and neck cancer · Survival

Introduction

Head and neck cancer forms the major burden of cancer in the developing countries. Head and neck cancer accounts for around 30% of all cancers in males as per recent consolidated report of hospital-based cancer registry of NCPR.

Most of the head and neck cancer patients present with locally advanced stage which requires multimodality treatment. Concurrent chemoradiation is the standard of care and is well supported by various clinical trials. Despite advancement in the treatment approach of head and neck cancer in terms of surgery, chemotherapy, and radiotherapy, overall long-term survival remains low due to uncontrollable persistent and recurrent disease [1, 2]. This low survival rate has demanded the need for newer treatment approaches and prognostic markers.

Tissue biomarkers like HPV, P53, and EGFR status recently found to have potential new modalities for diagnosis, treatment, and for predicting clinical outcome.

In our previous study "impact of molecular predictors on the response rates in head and neck Cancer patients" by Koushik et al. [3] assessed the impact of molecular markers like HPV, P53, and EGFR status along with other prognostic factors like tobacco use, age, sex, and socioeconomic status on response to treatment of head and neck cancer patients. Our present study intended to provide update of the impact of those molecular markers on survival.

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Methods and Materials

Study Design: Prospective cohort study

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

Objective: To correlate the HPV, EGFR, and P53 status with the survival rate

Inclusion Criteria:

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

Exclusion Criteria:

- Post-op cases

Methods

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
- PCR technique used for EGFR status detection

P53 Testing

- P53 testing also done on paraffin embedded histopathological blocks
- PCR technique used for p53 mutation analysis

Treatment Given

- All the patient were treated with external radiation to a dose of 66 Gy in 33 fractions along with concurrent weekly cisplatin chemotherapy at a dose of 40 mg/m^2.

Response Assessment

- Assessment based on RECIST criteria
- Correlation of HPV, EGFR, and p53 status on response

Survival Analysis

- 3-year minimum follow-up and analysis have been done.

Statistics

- All statistical analyses were done on IBM SPSS v 20.0 software
- Mean and standard deviation was applied for nominal variables
- Pearson's chi-square test was applied for analysis of variance

Results

Subsite-wise distribution of the molecular markers in head and neck cancer patients

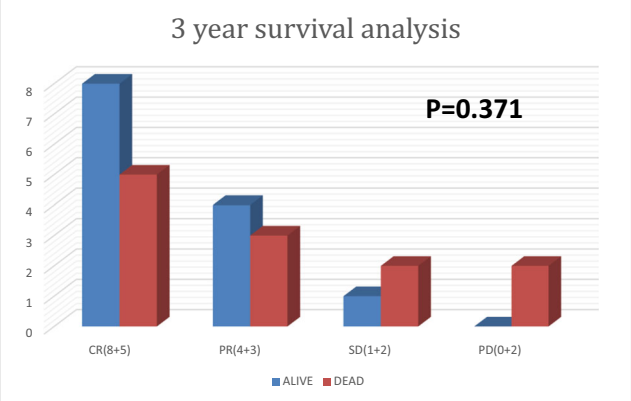
Site	Total number		HPV positive		EGFR positive		P53 positive	
Oral cavity	4	16%	1	14.2%	4	19.04%	4	21.05%
Oropharynx	4	16%	3	42.8%	11	57.89%	7	36.84%
Hypopharynx	1	4%	0	0%	1	4.76%	1	5.26%
Larynx	7	28%	2	28.5%	4	19.06%	6	31.57%
Nasopharynx	2	8%	1	14.2%	1	4.76%	1	5.26%

Stage-wise 3-year survival of head and neck cancer patients

Stage group	Total number	Survival	
		Alive	Dead
I	2	2	0
II	1	1	0
III	9	6	3
IV	13	6	7
Total	25	15	10

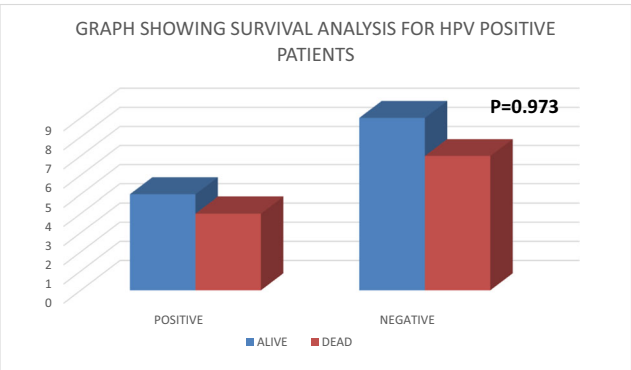
Three-year overall survival of the head and neck cancer patients

Response	Survival		Total
	Alive	Dead	
CR	8	5	13
PR	4	3	7
SD	1	2	3
PD	0	2	2
Total	13	12	25



Three-year survival of HPV-positive patients

HPV status	Alive	Dead	Total
Positive (7)	4	3	7
Negative (18)	10	8	18
Total	13	12	25

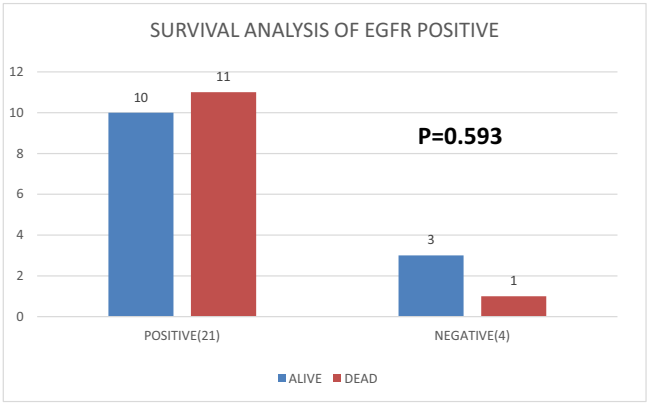


Only 57.14% (4) of HPV-positive patients are alive at 3 years with *p* value 0.973.

Three-year survival of EGFR-positive patients

EGFR status	Alive	Dead	Total
Positive (21)	10	11	21
Negative (4)	3	1	4
Total	13	12	25

Graph showing three-year survival analysis of EGFR positive patients

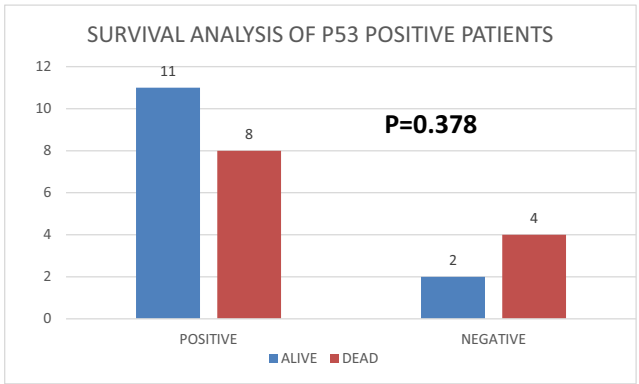


Only 47.61% of EGFR-positive patients are alive after 3 years whereas 75% of EGFR-negative patients are alive with *p* value 0.593.

Three-year survival analysis of P53 positive patients

p53 status	Alive	Dead	Total
Positive	11	8	19
Negative	2	4	6
	13	12	25

Graph showing three-year survival analysis of p53 positive patients



57.89% of p53-positive patients are alive when compared to 33.33% survival of p53-negative patients with *p* value 0.378.

Discussion

There are many studies which are showing the association between HNSCC with HPV infection, p53 mutation, and EGFR expression.

It has been found that HPV infection is independently associated with the pathogenesis of HNC [4]. The pathogenesis behind the HPV infection and head and neck cancers is through the viral oncoprotein E6 and E7; these proteins degrade the p53 and interfere with RB function leading to upgradation of $P16^{INK4a}$ by the loss of negative feedback control [5]. The loss of p53 and RB gene leads to uncontrolled cell cycle progression which leads to the progression of disease [6]. Increased expression of P16 protein has been demonstrated in HPV-related HNC and cervical cancer [6, 7]. And in those trials, it has been shown that P16 expression may serve as a surrogate marker of oncogenic HPV infection in predicting HPV-related tumors [8, 9]. Hence, in our study, P16 is used to assay the HPV infection.

In our study, there was no statistical significance of the P16 status on 3-year survival but the P16-positive patients showed better survival (57.14%) compared to P16-negative patients. In a study by K Kian Ang et al., they found that patients with p16-positive oropharyngeal carcinoma (OPC), compared with patients with p16-negative OPC, had better 3-year probability of PFS (72.8% vs 49.2%, respectively; p 0.001) and OS (85.6% vs 60.1% p 0.001), but tumor epidermal growth factor receptor (EGFR) expression did not distinguish outcome [2]. Many trials have showed better survival in HPV-positive HNC [10] as well as P16-positive tumor [7, 9, 11].

The role of p53 as a prognostic marker of squamous-cell carcinoma of the head and neck is controversial. Many of the trial showing decreased survival associated with P53 mutation [7, 8] and few other studies showed no correlation [12].In a study by M luana et al. found that P53 mutation in head and neck cancer associated with reduced survival. In this study, the median survival was 3.2 years among patients with a P53 mutation and 5.4 years among patients with wild-type tumors ($p = 0.009$). Five-year overall survival was reached in 40.7% of patients with P53 mutations and in 54.8% of patients with wild-type P53 ($p = 0.009$) [13]. In a study by Jenni K Peltonen et al. showed P53 mutation correlated more with environmental factor like prolonged tobacco exposure and P53 mutation is more common in younger age [14].

In our study, 57.89% of patients with P53 mutation are alive after 3 years compared to 33.33% of P53-negative patients even though which was statistically not significant. But it is contrary to many literatures published. The cause could be contributed from other multiple factors, and further analysis of P53 in the survival of head and neck cancer is required.

FGFR expression has been associated with poor survival in various trials. In our study, 47.61% of EGFR-positive patients are alive at 3 years compared to 75% of EGFR-negative patients. In one prospective study, EGFR expression is quantitatively evaluated by SAMBA system, and they found that the OS and DFS rates of patients with high EGFR status on survival are lesser significant, and the local relapse was significantly higher ($p = 0.0031$) proving EGFR expression to be a strong independent prognostic marker in head and neck cancer [2].

In a series of patients with HNSCC underwent randomization to receive accelerated radiotherapy vs conventional fractionation found that independent of treatment modality, high EGFR expression was associated with higher local recurrence ($p = 0.0163$) and overall survival ($p = 0.0010$) [15]. These trials showed that EGFR expression when evaluated quantitatively proved to be a prognostic marker associated with poor outcome in HNSCC treated with radiotherapy.

Conclusion

The result of our study is in concordance with the literatures published, even though statistically not significant but P53 mutation in HNC in our observation showed better survival compared to wild type which is in contrary to the many studies published, and our study also showed that the response to the treatment is more importantly associated with survival.

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