Original Article

The Association of Human Papillomavirus Infection in Indian Cohort of Head-and-Neck Squamous Cell Cancer Patients and its Impact on Treatment Outcome

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Abstract

Context: Head and Neck Squamous Cell Carcinomas (HNSCCs) patients with HPV-infected tumors have more favorable prognosis, however data is very sparse in Indian literature. **Aim:** Our study aims to detect p16 (a surrogate for tumor HPV DNA) in HNSCC and its effect on the survival of the patients. **Settings and Design:** Observational (prospective study). **Materials and Methods:** This study was conducted amongst 50 cases of HNSCC. All tissue samples for biopsy were subjected to Immunohistochemistry to study p16 expression, a surrogate marker for HPV. The patients were treated by Radiotherapy or concurrent chemo-radiotherapy depending on performance status and stage of disease. Evaluation was done at 3, 6, 12, 18, and 24 months after treatment. Survival analysis was used to check the outcome of Radiotherapy using Kaplan Meyer survival curves and cox proportional hazards model. **Results:** Majority of patients had Stage III disease (33 patients – 66%). 16 (32%) patients were HPV positive and 34(64%) were negative. Out of the 16 HPV positive cases, majority, 15 (93.75%) cases were associated with oropharyngeal carcinoma. 2 year DFS for HPV positive was 84% compared to 58% in HPV negative patients (P = 0.089) and 2 year overall survival for HPV positive patients was 83% compared to 52% for HPV negative patients (P = 0.03). **Conclusions:** Our study concluded that 32% of the HNSCC patients were positive for HPV. Patients who were HPV positive had better disease free survival and overall survival.

Keywords: *Head-and-neck cancer, human papillomavirus, radiotherapy*

Introduction

Head-and-neck squamous cell carcinoma (HNSCC) is the sixth most common cancer and the eighth most common cause of cancer deaths worldwide. Its incidence varies widely among different regions.[1] In India, HNSCC is one of the most common causes of cancer-related morbidity and mortality with an incidence of 12% of all cancers.[2]

HNSCCs are characterized by multiphasic and multifactorial etiopathogenesis. Although tobacco and alcohol consumption are the most common risk factors for head-and-neck malignancy, the role of human papillomavirus (HPV), a DNA virus has recently been implicated in the initiation and development of these lesions.

Recent studies have indicated that in HNSCC, patients with HPV-infected tumors have a more favorable prognosis as compared with patients whose tumors are HPV negative.[3-6] HPV positivity is associated with lower exposure to tobacco and alcohol and with younger age at time of diagnosis. These factors may by themselves positively influence the prognosis regardless of tumor biology.

The determination as to whether a patient's HNSCC is HPV induced or not is generally done in two ways-either by the detection of HPV DNA through polymerase chain reaction or through the utilization of immunohistochemistry. In general, immunohistochemical (IHC) staining of tumor p16 expression has gained broad acceptance as a biomarker of infection with HPV in HNSCC. A high correlation between HPV and p16 expression in HNSCC,[7-10] particularly oropharyngeal carcinomas, has consistently been reported.

The management of patients with HNSCC involves multimodality treatment surgery, radiotherapy, and concurrent chemotherapy. Chemoradiotherapy is an important therapeutic modality and is used either as adjuvant or primary treatment modality.[11]

How to cite this article: Singh S, Nautiyal V, Chauhan N, Gupta M, Ahmad M. The association of human papillomavirus infection in Indian cohort of head-and-neck squamous cell cancer patients and its impact on treatment outcome. Clin Cancer Investig J 2017;6:247-53.

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Access this article online

Website: www.ccij-online.org

DOI: 10.4103/ccij.ccij_62_17

Quick Response Code:

It has been proposed that the superior treatment response observed for patients with HPV-positive tumors could be attributable to an intrinsic-enhanced radiosensitivity of these tumors. Indeed, there has been a recent focus for dose de-escalation for HPV-positive HNSCC.[12-14]

HNSCC is predominantly a loco-regional disease, and achieving local tumor control is essential for survival.[15] Although various modification of treatment options has been done, individual patients with HNSCC still show considerable variation in clinical outcome. This indicates that other factors defining tumor response are yet to be discovered[16,17] and HPV infection may be one such factor.

There are robust data on the prevalence and outcome in HPV-positive HNSCC, especially oropharyngeal carcinoma from Europe and US, but in India, published literature is very sparse.

Thus, the study aims to observe the association between HPV in HNSCC and its impact on treatment outcome in our population. The study was an observational, prospective, noninterventional type, as the patient's treatment was not altered on the basis of p16 positivity.

Materials and Methods

This study was conducted after ethical clearance from institutional ethics committee and written informed consent was obtained from all patients.

Study design

It was an observational (prospective study) with a total of 50 cases of HNSCC, which were treated from June 2014 to January 2016 by radiotherapy or chemoradiotherapy.

Selection of subject

Patients with newly diagnosed, nonmetastatic squamous cell carcinoma of head-and-neck cancer, treated by radiotherapy with or without concurrent chemotherapy, were included. Those patients, who were previously treated by any sort of oncological treatment such as surgery/chemotherapy/radiotherapy, any other malignancy in head and neck or second malignancy or those with concomitant comorbid conditions, which precluded the use of radiotherapy, Eastern Cooperative Oncology Group performance status (ECOG PS) score ≥3, and major medical or psychiatric illness, which may interfere with either completion of therapy or follow-up were excluded.

Study protocol

- All included HNSCC patients were tested for HPV positivity. The definitive treatment modality was decided by tumour-specific factors-operability, stage of the disease, and general condition of the patient. Pretreatment workup included detailed ear, nose, and throat examination, laryngopharyngoscopy, routine blood counts, liver and renal function tests. Imaging such as chest X-ray/contrast-enhanced computed tomography (CECT) thorax, ultrasonography abdomen, CECT, and/or magnetic resonance imaging neck was done as and when indicated
- Biopsy samples were taken from each patient and histopathological confirmation and grading of the lesions was also done
- Evaluation of HPV Status: The tissue was subjected for immunohistochemistry to study p16 expression, which is a surrogate marker for HPV. IHC staining was carried out with p16 rabbit/mouse monoclonal ready to use antibody (CD INK4a) (Biogenex) using epitope retrieval technique. The standard protocol included deparaffinization, rehydration, and incubation with primary antibody and further treatment with high sensitivity polydetector horseradish peroxidase/diaminobenzidine system (Bio-SB). IHC expression for p16 was graded weak, moderate, or strong according to nuclear and cytoplasmic staining intensity using clinically established criteria[18]
- The patients were treated by radiotherapy alone or concurrent chemoradiotherapy depending on PS and stage of disease.

Evaluation was done at 3, 6, 12, 18, and 24 months after the completion of treatment.

Data management and statistical analysis

A database was constituted using available software solutions SPSS Inc., SPSS for windows, Version 16.0, Chicago, USA and electronic spreadsheets (MS Excel) to store and manage the collected data.

Qualitative Data was expressed in terms of frequency/percentage. Parametric and nonparametric test was used to determine the level of significance for categorical variables. Survival analysis was used to check the outcome of Radiotherapy using Kaplan–Meier survival curves and Cox proportional hazards model. Value of $P < 0.05$ was considered statistically significant.

Results

Fifty patients were recruited in the study, and median follow-up was 27 months by reverse KM. Median age and sex did not differ significantly between the groups, and majority of patients in all subsites were diagnosed in disease Stage III or IV. Demographic characteristics of the patients are shown in Table 1.

Among 16 HPV-positive patients, 4 (25%) were nonsmokers, 6 (37.5%) patients were >10 packs/year smokers, and remaining 6 (37.5%) were <10 packs/year smokers. As per ECOG Scoring criteria, majority of patients were having good PS, that is, I and II being (48) 96%.

Median radiation dose delivered was 66 Gy/33# @2 Gy/# in 5 days a week over 6–7 weeks using conventional, 3DCRT, or IMRT techniques.

Among 50 patients, 36 (72%) patient received concurrent chemotherapy with injection cisplatin (35 mg/m^2) weekly; rest of the patients did not receive concurrent chemotherapy in view of early disease, old age, or poor PS.

A total of 44 (88%) patients completed the chemoradiotherapy/radiotherapy treatment (88%) in scheduled time. In 6 (12%) patients, radiotherapy was interrupted due to toxicity of treatment and social problems, out of which 4 (8%) received it for <1-week duration. A total of 26 (52%) patients required Ryle's tube insertion during the treatment for maintaining nutrition.

Majority of the patients had Grade II toxicity 24 (48%) followed by Grade I toxicity 17 (34%), respectively, Grade III skin reactions were observed in 9 (18%) patients only.

Mucositis Grade II and Grade III were observed in 33 patients (66%) and 14 patients (28%), respectively. Grade IV toxicity was observed only in 3 patients (6%), who required treatment interruption.

A total of 29 (58%) patients were disease-free, 11 (22%) patients had disease recurrence, 9 (18%) patients were found to have residual disease, and only one patient had distant metastasis.

HPV status of patients, done by IHC technique, shows 16 (32%) positive cases and 34 (64%) negative cases. In 16 HPV-positive patients, 15 (93.75%) had oropharyngeal, and one (6.25%) patient had hypopharyngeal primary.

Outcome

Tables 2 and 3 depicts stage-wise HPV status and survival.

Disease pattern among HPV-positive group shows that 12 (75%) patients were disease-free. Two (12.5%) patients presented with local failure and 2 (12.5%) cases were lost to follow-up. Among HPV-negative group, 15 (44%) patients were disease-free, 17 (50%) patients had residual disease and local failure. One patient each (6%) had distant failure and lost to follow-up.

Figure 1 depicts stage-wise KS survival curve and Figure 2 depicts KS survival according to HPV Status.

Among 16 HPV-positive cases, 12 (75%) patients were alive and 2 (12.5%) got expired. Among 34 HPV-negative cases, 25 (73%) patients were alive and 9 (27%) were expired.

In the final Cox proportional hazards analysis with overall survival (OS) as the end point, survival has decreased as the stage was increasing, Stage I, (hazard ratio [HR], 0.00; 95% confidence interval [CI], 0.00), Stage II (HR, 0.3; 95% CI, 0.039–2.32), Stage III (HR, 0.811; 95% CI, 0.301–2.18), p16INK4A expression (HR, 0.23; 95% CI, 0.054–1.04), respectively were independent factors and were associated with a good prognosis.

Table 1: Demographic profile of the patients at baseline (*n*=50)

	Frequency (%)
Age groups	
30-40	2 (4)
41-50	6 (12)
51-60	20 (40)
61-70	16 (32)
71-80	6 (12)
Tobacco habits	
Tobacco users	46 (92)
Nontobacco users	4 (8)
Sex	
Male	46 (92)
Female	4 (8)
ECOG PS	
I	7 (14)
II	41 (82)
III	2 (4)
Site	
Hypopharynx	6 (12)
Larynx	15 (30)
Oropharynx	29 (58)
Stage	
I	2 (4)
II	12 (24)
III	33 (66)
IV	3 (6)
HPE	
MDSCC	34 (68)
PDSCC	11 (22)
WDSCC	5 (10)
HPV	
Negative	34 (68)
Positive	16 (32)
Radiation details	
Completed scheduled dose	44 (88)
Concurrent chemotherapy (cisplatin)	
Yes	36 (72)
No	14 (28)
Total	50 (100)

HPV: Human papillomavirus, MDSCC: Moderately differentiated squamous cell carcinoma, PDSCC: Poorly differentiated squamous cell carcinoma, WDSCC: Well-differentiated squamous cell carcinoma, HPE: Hepatoportoenterostomy, ECOG PS: Eastern Cooperative Oncology Group performance status

Discussion

HNSCC patients showed poor survival; therefore, we need good biomarkers to identify patients who may benefit from chemoradiotherapy/radiotherapy and to predict their long-term survival. There has been a surge of interest in trying to identify and classify biomarkers, which can help the clinicians to prognosticate the patients in a better way. We found that 92% of patients were male and 8% were female which shows high prevalence of

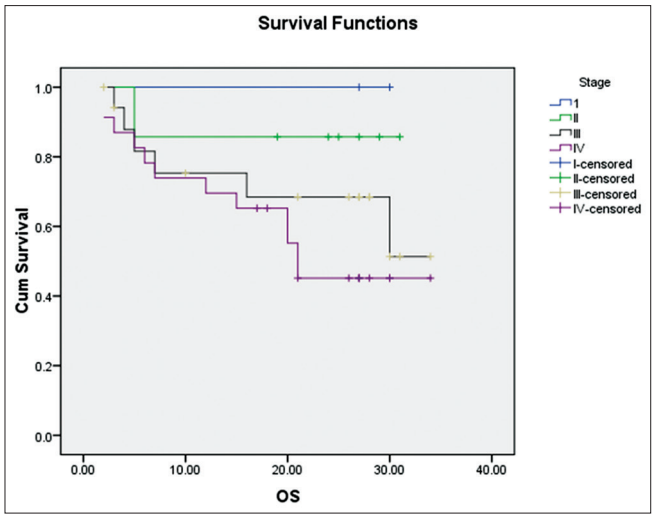

Figure 1: Kaplan–Meier estimate stage-wise overall survival

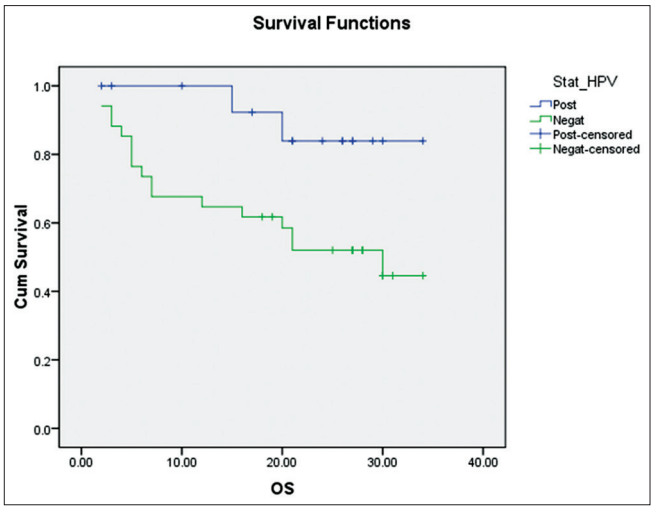

Figure 2: Kaplan–Meier estimate overall survival in both human papillomavirus positive and negative patients

Table 2: Stage-wise distribution according to human papillomavirus status

Stage	HPV status		Total
	Positive	Negative	
I	0	2	2
II	3	4	7
III	8	10	18
IV	5	18	23
Total	16	34	50

HPV: Human papillomavirus

Table 3: Stage-wise overall survival

Stage	Total	Events	Censored	Percentage
I	2	0	2	100
II	7	1	6	85.7
III	14	5	9	64.3
IV	20	9	11	55
Overall	43	15	28	65.1

head-and-neck cancer in males, which was consistent with the study conducted by Gaur *et al*. which showed high male-to-female ratio (11.5:1).[19] It was further noted that almost half (50%) of the patients were in the age group of 51–60 years, which is quite similar to study done by Mehrotra *et al*.[20] where comparison of the age-specific prevalence rates of head-and-neck cancer was highest in patients belonging to 50–59 years age group. Chaturvedi *et al*. also observed that 56% of patients were among the age group 51–60 years.[21] Various studies from west showed that patient with HPV +ve HNSCC were seen in young males with the median age varying from 54 to 57 years.[22,23] In our study, 62% of HPV-positive patients were among 50–60 years of age group. Our study had only 8% females and none was positive for HPV. This can be explained by the differences in the pattern of tobacco use and sexual behavior in females in India compared to the US as p16 positivity is likely to be detected among the nonsmokers and those with high-risk sexual behaviors.[24,25] The high prevalence of tobacco use (92%) in this study has been supported by a large series of oropharyngeal carcinoma from India with the prevalence of tobacco use of 80.5%.[26] HPV positivity is common in nonsmokers and nondrinkers in HNSCC, the degree of synergism of HPV infection with the classical risk factors is not clear, and data exist for both synergistic[27] and additional effect.[28] We observed that oropharyngeal carcinoma constituted 93.8% of HPV-positive cases which was similar to a study done by Ang *et al*., in which 96.1% of HPV-positive patients were of oropharyngeal carcinoma.[29] It has been widely accepted that smoking and tobacco chewing habit are established risk factors for head-and-neck carcinomas.[30] About 86% of patients were tobacco users. Most common symptoms at presentation was odynophagia, which was seen in 64% of the patients followed by difficulty in swallowing and change in voice which coincides with the usual clinical symptoms of patients with head-and-neck carcinomas.[31] On subsite analysis, oropharyngeal site accounted for 58% of HNSCC followed by larynx (30%) and hypopharynx (12%), which was comparable with a study conducted by Ang *et al*.[29] which also reported that among the various sites in the head-and-neck region oropharyngeal malignancies constitutes 45.32%. Similar results were also reported in a study done by Pandey *et al*. in Uttarakhand region which showed that oropharyngeal cancers constitute 33.24% of head-and-neck malignancies.[32] Seventy-two percentage of the patient population presented with Stage III and IVa disease, which was comparable to the patient population in the studies done by Agarwal *et al*., which reported about 70%–75% cases of head-and-neck cancer presented in a locally advanced stage with a significant proportion in an inoperable stage.[33] Various studies have shown that classical HPV-positive patient has a small tumor size with

large multiple metastatic cystic nodes.[21,34-36] Although there is no robust data in correlation of HPV/p16 status and tumor characteristics.[37,38] In this study, although the p16-positive patients did show a trend toward having small tumor size and node positivity, it did not reach statistical significance, due to the small number of p16-positive tumors. On histopathological examination of the biopsy specimens, it was found that maximum number of patients had moderately differentiated histology 34 (68%) followed by poorly differentiated 11 (22%) and well-differentiated squamous cell cancer (SCC) 5 (10%). These observations were not consistent with findings of Weinberger *et al*. which showed that maximum number of patients had well-differentiated histology.[5] In our study, 32% patients were positive for HPV. In India, there are some series of head-and-neck SCC patients showing a prevalence of HPV ranging from 17% to 50%.[39-42] Studies from the West shows a higher p16 positivity rate of about 43%–66%, especially in oropharyngeal carcinoma.[43,44]

At the time of study, 76%[37] of patients were alive. On further analysis, 66% of them were alive without disease and 34% were alive with disease.

In our study, HPV was detected in the patients by IHC for p16, which has been established as a reliable surrogate marker for depicting HPV status.[18]

On doing IHC analysis of the patients for HPV infection, 32% of the patient population tested positive for HPV infection which was comparable to study done by Jalouli *et al*. which demonstrated 35% HPV positivity in head-and-neck SCC patients.[45]

Lassen *et al*. showed that 21% of female population tested positive for HPV; however, we could not found any HPV positivity in females.[46]

The 2-year OS for HPV-positive patients was 83%, which was found to be significant, compared to 52% for HPV-negative patients (P = 0.03). Ritchie *et al*. also reported an OS benefit for HPV-positive head-and-neck Cancer patients (71% vs. 49%).[47] Similar results were also reported by Licitra *et al*. who showed that HPV-positive patients had a better OS as compared to patients with HPV negative (79% vs. 46%).[4]

Two-year disease-free survival (DFS) for HPV positive was 84% when compared with 58% in HPV-negative patients (P = 0.089), corroborative findings were related by Lassen *et al*. who showed that HPV-positive patients had better treatment outcome with DFS of 72% in HPV-positive patients versus 34% in HPV-negative patients.[38] Nichols *et al*. also reported 3 year OS of 89% versus 65% (P = 0.0005) and 3 year DFS of 77% versus 45% (P = 0.02) in a case series of 44 patients, which is comparable with our study.[48]

Strength and limitation

The strength of our study is that, this is the first study of its kind in Uttarakhand region, and hence, it will help to form a baseline for the future studies in this region. The limitation of this study is its small sample size. The lack of data on the high-risk sexual behavior and its relation with the p16 prevalence is yet another limitation of our study. Further large-scale studies with a longer follow-up are required to make definite association of HPV-positive cases and other prognostic factors in head-and-neck cancers.

Conclusion

HNSCC continue to have poor survival, despite advances in treatment modalities. In recent years, infection with high-risk HPV has been implicated in the pathogenesis of HNSCC.

On the basis of observations and their analysis, we concluded that HPV was positive in 32% of the patient's population and it was most associated with carcinoma oropharynx among all anatomical subsite of head-and-neck cancer. Patients who were HPV positive had a significant better OS and DFS as compared to patients who were negative.

HPV infection has established its role as a marker for increased response to chemoradiotherapy/radiotherapy, and in turn, leads to increased OS and DFS. We, thus, believe that p16 or HPV-DNA testing should be routinely recommended for HNSCC patients, especially when smoking and alcohol use is not suspected.

We also recommend adequately powered studies with large sample sizes, so that multivariate analyses can be conducted to account for multiple co-existing prognostic and predictive factors.

Acknowledgment

The authors are highly thankful to SRH University for permitting this research study and for providing all assistance for the same.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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