Original Article

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A Study on Prevalence of HPV Infection in Head and Neck Cancer

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Abstract

Introduction: The HPV prevalence in India ranges from 33.6% in the Eastern region to 67% in South India and 15% in Western India. The prevalence of HPV6, HPV11, HPV16, and HPV18 were 13%, 20%, 42%, and 47%, respectively. HPV16 was most common, followed by HPV18 and then cross infection (16 and 18); 41% of patients had multiple HPV infections. *Methodology:* A total of 50 patients with histo pathologically proven head and neck cancer were enrolled into the study. All the patients were given concurrent chemo irradiation after fulfilling the inclusion / exclusion criteria. Patients were enrolled into the study after basic investigations such as CBP, RFT, histopathology and metastatic work-up with chest radiograph and ultrasound of abdomen was performed and found to be within normal limits. *Results*: Out of 50 patients 15 were positive for HPV (P16) and 35 were negative. Prevalence of HPV in HNSCC was 30%. All HPV positive patients were allotted to Arm A and rest of patients were Arm B. *Conclusion*: No significant association was found between habits like smoking, alcohol and betel nut chewing and HPV infection.

Keywords: HPV Infection; Head and Neck Cancer; Smoking.

Introduction

Head-neck cancer is a broad term referring to the heterogeneous group of malignant neoplasm's arising in the head-neck region. Commonly, the term head-neck cancer refers to cancers of the upper aero digestive tract, which arise from the epithelial lining of the oral cavity, pharynx and larynx.

Cigarette-smoking and alcohol consumption are the main risk factors for HNSCC in the Western population, whereas the use of smokeless tobacco and areca nut are the most common risk factors for HNSCC in Southeast Asia [1].

Although tobacco and alcohol consumption are the primary risk factors for development of head and neck squamous cell carcinoma (HNSCC), high-risk human papilloma virus (HR-HPV) is etiologically linked to a subgroup of cancers in the head and neck region [2]. The overall prevalence of HPV in HNSCC ranges from 5% to 75% depends upon population studied and with highest prevalence in the tonsils and the base of the tongue [3]. HPV can possibly also cause non oropharyngeal squamous cell carcinoma as oral cavity (23.5%) and larynx (24%) reported in meta-analysis by Kreimer et al 2005 [3], causation remains unestablished. Of oropharyngeal squamous cell carcinoma (OPSCC), 38-44.8% is estimated to be related to HPV infection, generally to HPV16 (>80%) and less generally to HPV18, 31, or 33. These are the same oncogenic viruses that induce anogenital cancers [4].

The HPV prevalence in India ranges from 33.6% in the Eastern region to 67% in South India and 15% in Western India [5,6]. The prevalence of HPV6, HPV11, HPV16, and HPV18 were 13%, 20%, 42%, and 47%, respectively [5]. HPV16 was most common, followed by HPV18 and then cross infection (16 and 18); 41% of patients had multiple HPV infections.Lesions of the tongue had the highest rate (9 of 11) of HPV infection.

Advanced stages (III, IV) had higher infection rates of HPV as compared to earlier stage [7].

Despite of advanced stage at presentation, HPV positive HNSCC have improved loco regional response to RT alone or radio chemotherapy and

improved disease free survival and overall survival especially in oropharyngeal squamous cell carcinoma [18,19]. Improved prognosis of HPV associated oropharyngeal cancer was well established and effect of HPV on other non oropharyngeal cancer is still ambiguous.

Meta analysis by Ragin and Taioli suggested that HPV has no effect on overall survival and disease free survival in non oropharyngeal cancer. The effect of HPV on head and neck cancer was less explored in India where oral cavity cancers are more common than western countries. The effect of HPV on clinico-pathological features and treatment outcome was not well established in Indian population [8].

HPV DNA can be directly assayed in biopsies by polymerase chain reaction (PCR) or in situ hybridization(ISH). The p16 expression was highly correlated with thepresence of HPV-DNA and can be used as a surrogate marker for HPV-positivetumor. Greater than 86% of HPV-associated tumors over express p16INK4A (p16), acyclin-dependent kinase inhibitor, and only 3% of HPV-non associated tumors over express p16.

In this study, HPV in tumor tissue samples were detected with the help of p16 immuno-histochemistry (IHC). Immunohistochemical detection of p16 expression isused as a surrogate for HPV detection, because-p16 expression was shown to be significantly associated with the presence of HPV, and it is an inexpensive, simple procedure when compared with HPV detection techniques like PCR and ISH. Using a surrogate marker such as p16 to evaluate HPV status in HNSCC patients is especially significant in developing countries where HPV sequencing or ISH may not be affordable [8].

Methodology

A total of 50 patients with histo pathologically proven head and neck cancer were enrolled into the study. All the patients were given concurrent chemo irradiation after fulfilling the inclusion / exclusion criteria.

Inclusion Criteria

1. ECOG: 0-2 (ANNEXURE-II)
2. Age > 18 to <80 yrs
3. Locally advanced HNSCC
4. Previously untreated HNSCC

Exclusion Criteria

1. ECOG 3-4
2. Stage I /IV C
3. Evidence of distant metastasis at presentation
4. Second primary cancer after initial curative therapy

Patients were enrolled into the study after basic investigations such as CBP, RFT, histopathology and metastatic work-up with chest radiograph and ultrasound ofabdomen was performed and found to be within normal limits.

Results

A total of 50 patients of head and neck squamous cell cancer(HNSCC) were enrolled in this study and analyzed. All patients underwent baseline evaluation as per protocol. HPV status was evaluated with the help of P16 immunohistochemistry.

Treatment consisted of conventional RT [6600cGy / 33fractions 200cGy / fraction over 6.5 to 7 weeks] with concurrent injection Cisplatin (40 mg/ m^2/ week). During concurrent chemo radiation patients were evaluated for complete tumor and node response. After completion of the treatment, the end response was noted by assessing the primary tumor and node response by WHO criteria and first follow up response assessment was done at two months after treatment.

In the study of 50 patients of HNSCC, 2 patients were expired during the second week of treatment(one patient was due to myocardial infarction and another due to pneumonia), 6 patients were lost follow up after completion of treatment and 12 patients were absconded during the treatment. These 20 patients were excluded from the final analysis of loco regional control which was the end point of this study.

A prospective clinical study with 50 patients of STAGE II – STAGE IV B cancer were taken to assess the incidence of HPV (P16) , clinic pathological features and loco regional response to therapy. Common habits found in this study were smoking, alcohol consumption and betel nut / gutka chewing.

They were divided into 3 groups based on number of pack-yrs i.e. never smoked, ≤ 10 pack yrs and >10 pack yrs which included in Arm A 4(26.6%), 8(53.3%) & 3(20%) patients respectively. Similarly in arm B there were 17(48.5%), 15(42.8%) & 3(2. 8%) patients respectively.

In arm A, 79.9% of patients were never smoked and ≤ 10 pack yr. In arm B, 91.3% of patients were never smoked and ≤10 pack yr . The chi square ($\chi 2$)

value for significance of correlation between groups was 2.593 and P value was ≥0.05. With respect to smoking habit, the difference between arm A and arm B was not significant.

They were divided into 3 groups based on duration of alcohol intake history i.e. never, ≤10 yrs and >10 yrs which included in Arm A 12(80%), 1(6.6%)& 2(13.3%) patients respectively. Similarly in arm B there were 22(68.8%), 10(28.5%) & 3(8.5%) patients respectively.

80% (12/15) of patients in arm A had no history of alcohol consumption and 68.8% of arm B patients had no history of alcohol consumption. The chi square ($\chi 2$) value for significance of correlation between groups was 2.982 and p value was ≥0.05. In consideration to alcohol habit, the difference between arm A and arm B was not significant.

In arm A 40% (6/15) of HPV positive patients had the habit of betel nut chewing present.

Table 1: Number of patients with H/O smoking in Arm A and Arm B patients chi square and P value

Smoking Pack Years	N=50		Chi-Square $\chi 2$	P
	ARM A	ARM B		
Never smoked	4(26.6%)	17(48.5%)	-	-
≤10 Years pack	8(53.3%)	15(42.8%)	2.593	0.273
>10 Years pack	3(20%)	3(2.8%)	-	-
Total	15(100%)	35(100%)	-	-

Table 2: Number of patients with H/O alcohol in Arm A and Arm B patients, chi square value and P value

Alcohol Years	N=50		Chi-Square $\chi 2$	P
	ARM A	ARM B		
No	12(80%)	22(68.8%)		
≤10 Years	1(6.6%)	10(28.5%)		
>10 Years	2(13.3%)	3(8.5%)	2.982	0.225
Total	15(100%)	35(100%)		

Table 3: Number of patients with H/O betel nut chewing in Arm A and Arm B patients, chi square value and P value

Betel-Nut Chewing	N=50		Chi-Square	P
	ARM A	ARM B		
Yes	6(40%)	14(40%)		
No	9(60%)	21(60%)	0.000	1.000
Total	15	35		

In arm B 40% (14/21) HPV negative patients had the habit of betel nut chewing.

The chi square $\chi 2$ value for significance of correlation between groups was 0.000 and p value was ≥ 0.05.With respect to habit of betel nut /gutka chewing, the difference between arm A and arm B was not statistically significant.

The following observations were made.

Out of 50 patients 15 were positive for HPV (P16) and 35 were negative. Prevalence of HPV in HNSCC was 30%. All HPV positive patients were allotted to Arm A and rest of patients were Arm B.

Arm A	Arm B
HPV/P 16 POSITIVE N=15 (30%)	HPV/P 16 NEGATIVE N=35 (70%)

In arm A: Age distribution was 20-30yrs -0%, 30-40 yrs 4 (26%), 40-50 yr –4(26%), 50-60 yrs- 3(20%), above 60 yrs were 4 (26%).

In Arm B: Age distribution was 20-30yrs -1(2.8%), 30-40 yrs-7 (20%), 40-50yr-8 (22.8%), 50-60 yrs 8 (22.8%), above 60 yrs were 11 (31.4%).

Mean age of presentation in arm A was 48.3 yrs with a standard deviation of 13.309.

Mean age at presentation in arm B was 51.37 yrs with a standard deviation of 14.061.

The patients in arm A were presented at younger age than arm B patients.The chi square value ($\chi 2$) for significance correlation among groups was 0.823 and p value was >0.05.

With respect to age, the difference between arm A and arm B was not statistically significant.

In Arm A, there were 15 patients, out of which 12 (80%) were males and 3 (20%) were females. In Arm B, there were 35 patients out of which 25 (71.4%) were male and 10 (28.6%) were female. In both arm A and arm B, majority of patients were male than female.

Table 4: Age distribution in Arm A and Arm B:

Age group	N=50		Chi square	P
	ARM A	ARM B		
20-30	0(0%)	1(2.8%)	-	-
30-40	4(26.6%)	7(20%)	-	-
40-50	4(26.6%)	8(22.8%)	-	-
50-60	3(20%)	8(22.8%)	0.823	0.935
Above 60	4(26.6%)	11(31.4%)	-	-
Mean age	48.3	51.37	-	-
Standard deviation	13.309	14.061	-	-

Table 5: Gender distribution in Arm A and Arm B

Gender	N=50		Chi-Square $\chi 2$	P
	ARM A	ARM B		
Male	12(80%)	25(71.4%)		
Female	3(20%)	10(28.6%)	0.401	0.527
Total	15(100%)	35(100%)		

Discussion

The present study was a prospective non randomized analytical study of patients with histo-pathologically proven squamous cell carcinoma of head and neck region, undertaken at Department of Radiation Oncology, Mehadi Nawab Jung Institute of Oncology & Regional Cancer Centre, Hyderabad. Fifty patients who were eligible for this study were planned to receive EBRT by conventional method using 2 parallel opposed fields to head and neck along with one low anterior field. Dose of 66 Gy along with concurrent chemotherapy with injection Cisplatin 40 mg/m2 weekly as a radiosensitizer was given as per protocol.

In many centers, p16 immunohistochemical staining is used as a surrogate marker for HPV for oropharyngeal cancer. However, it remains to be established whether p16 is a surrogate marker of the presence of HPV DNA in HNSCC. Despite the volume of literature on this topic, methodological differences between studies make comparisons of results difficult and any conclusions have to be interpreted with this caveat in mind.

P16 imunohistochemistry seems relatively cheaper when compared to HPV genotyping. Many studies on head and neck cancer have utilised p16 as a surrogate marker for HPV DNA.

Thus the present study aimed at determining the prevalance of p16 expression status in HNSCC as a surrogate marker for HPV & to determine clinico pathological features of HPV related HNSCC. It also aimed to study the influence of HPV/p16 status on loco regional tumor control after chemo -radiation in stage II-IVB HNSCC.

In Arm A, mean age at presentation was 48.3 yrs and in Arm B was 51.37 yrs. The mean age at presentation in arm A was less than arm B.

In Bahl A et al. study on north Indian population on prevalence of HPV in oropharyngeal cancers, suggested that patients who are HPV positive are younger compared to HPV negative cancer [9].

All these studies have demonstrated younger age at presentation in HPV positive patients than HPV negative. The present study results were consistent with above studies. But, the difference in age between HPV positive and HPV negative was not significant which was not consistent with above studies probably due to small sample size of the study.

In Arm A, there were 15 patients, out of which 12 (80%) were male and 3 (20%) were female. In Arm B, there were 35 patients out of which 25 (71.4%) were male and 10 (28.6%) were female. HPV positive tumors were common in males than females.

Lassen P et al analyzed the effect of HPV associated p16 expression onresponse to radiotherapy and survival in squamous cell carcinoma of head and neck. In this study HPV positive SCC common in males than females (66% vs. 34%) [10].

In prospective clinical trial conducted by Fakhry C et al 2008 on improved survival of patients with human papilloma virus – positive head and neck squamous cell carcinoma found that HPV positive tumors common in males than females (90% vs.10%) [11].

In a study conducted by Kumar et al 2007 on response to therapy in OPSCC in relation to biomarkers including HPV, EGFR, gender and smoking found that HPV positive OPSCC more common in males (22/30-73.3%) than females (5/12-41.6%) [12].

Smith et al 2010 analysis of HPV, p16 and p53 expression associated with survival of head and neck cancer found that HPV positive cancers were common in males than females (74.2% vs. 25.8%) [13].

HPV positive tumors were common in males (80%) than females (20%) in this present study. This result was consistent with above studies.

In arm A, never smoked were 4(26.6%), ≤10 pack yrs were 8(53.3%) and >10 pack yrs were 3(20%) patients. Similarly in arm B there were 17(48.5%), 15 (42.8%) & 3(8.5%) patients respectively. In this study, HPV positive HNSCC were common in never smoker and ≤ 10 pack yrs than >10 pack yrs similar to HPV negative patients, did not find any difference in smoking habit between arms.

They were divided into 3 groups based on duration of alcohol intake history i.e.-Arm A never 12(80%), d"10 yrs 1(6.6%) and >10 yrs 2(13.3%) respectively. Similarly in arm B there were 22 (68.8%), 10 (28.5%) and 3 (8.5%) patients respectively. In this study 80% of HPV positive patients were non alcoholic.

In arm A 9 (60%). In arm B in 21 (60%). the habit of betel nut chewing present in 6 (40%) patients and absent in the habit of betel nut chewing present in14 (40%) patients and absent In study by Gillison ML et al. on different risk factors in HPV positive and negative head and neck cancer found that upto 10-30% of HPV-positive head and neck squamous cell carcinomas were recorded in heavy tobacco and alcohol users [14]. This finding suggest that HPV-associated malignant disease not only arises in people who do not smoke or drink alcohol but also occurs in people with risk factors of tobacco and alcohol use.

In review analysis by woods RSR et al. on HPV related oropharyngeal SCC, suggested that in comparison to traditional HNSCCs, these patients are less likely to have excessive tobacco exposure and alcohol use, however HPV-related OPSCC do occur in those with tobacco exposure and alcohol use and in those without. It is highlyplausible that tobacco exposure potentiates the effects of HPV carcinogenesis but a role in the causation of HPV-related oropharyngeal SCCs has not been definitively determined from available evidence [15].

In a study by Stephen et al 2013 on significance of p16 on site specific HPV positive and HPV negative HNSCC found that there was no association between smoking history and HPV.

In recent study conducted by Bahl et al 2014 on prevalence and trends of HPV in OPSCC in north Indian population found no significant association between tobacco or alcohol consumption with HPV status [9].

In this present study, did not find any significant association when the habit of smoking, alcohol and tobacco/ betelnut chewing was correlated with HPV positive and negative status. Findings in this study was similar to that found in above mentioned study.

Conclusion

- Out of 50 patients, 15 patients were positive for HPV and 35 patients were negative for HPV.
- Prevalence of HPV in HNSCC was 30%.
- Mean age at presentation of HPV positive patients were 48.3 years and HPV negative patients were 51.3 years but the difference in age was not significant.
- Both HPV positive and negative cancers were more common in males than in females.

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