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Original Research Article

RISK PROFILE & CLINICAL PRESENTATION OF HPV POSITIVE VERSUS HPV NEGATIVE HEAD & NECK CANCERS

Dr. Neeti Verma[1] (Senior Resident), Dr. Rohit Sharma[2] (Professor and Head), Dr. Vinit Sharma[3] (Associate Professor) & Dr. Jaspreet Kaur[4] (Clinical Research Associate)

Department of ENT, Head Neck Surgery, Shri Ram Murti Smarak Institute of Medical Sciences, Bareilly, Uttar Pradesh[1,2&3]
Department of Biochemistry, SRMS-IMS, Bareilly, Uttar Pradesh[4]

Corresponding Author: Dr. Neeti Verma

ABSTRACT

BACKGROUND AND OBJECTIVES

Human papillomavirus (HPV) is established cause of head and neck cancers among other causes and is a better prognostic marker than tumor stage, which spurs clinically interest on HPV testing. The aims of our study were to find the incidence of HPV in various subsites of squamous cell carcinoma of Head & Neck cancers and to compare the risk profile and clinical presentation of HPV positive and HPV negative patients.

METHODS

Patients of suspected head neck cancers were biopsied. One biopsy sample was sent for histopathology, the second biopsy sample was preserved in NET (NaCl, EDTA, Tris) buffer at -20^{o}C temperature till biopsy report arrives. The patients who were confirmed of having squamous cell carcinoma were examined in detail for risk factors & clinical profile. Their second preserved samples were sent for HPV. All the HPV samples were processed in Real Time - PCR in The Central research Laboratory of the institute.

RESULTS

Comparison of risk profile and clinical presentation of HPV positive versus negative was done in terms of age, sex, habits, duration of symptoms, subsite, t stage, lymphnode status, clinical stage and histopathological type. Results showed risk factor significantly associated with HPV positives was abnormal sexual behaviour and majority of them presented early.

INTERPRETATION AND CONCLUSIONS

Abnormal sexual behaviour and early onset of symptoms were significantly associated with HPV16 positivity. Whereas there was no association between age, sex, T stage, nodal status, staging of cancer with HPV positivity.

KEYWORDS: HPV, Head Neck Cancers, Risk profile, Clinical presentation, NET Buffer, Prognosis, PCR, Squamous cell carcinoma

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1. Introduction

Head and neck Cancer are the sixth most common cancer in the world.[1] India has one of the world‘s highest incidences of head and neck cancers.[2] It arises from mucosal lining of the oral cavity, oropharynx, nasal cavity, nasopharynx, larynx, paranasal sinus, salivary glands. The most common, histological type is squamous cell carcinomas (SCC) and grade can vary from well differentiated keratinising to undifferentiated non keratinising.[3]

Head and neck SCC develops mostly via one of the two primary carcinogenic routes namely the chemical through exposure to tobacco and alcohol abuse which are known to be synergistic, and high risk HPV induced carcinogenesis.[4]

An increase in incidence of oropharyngeal squamous cell carcinoma— specifically oral cancers, has been seen in India, most notably in individuals aged 40–55 years. Unlike most tobacco-related head and neck tumors, patients with oropharyngeal carcinoma usually do not have a history of tobacco or alcohol use.[2] Instead, tumours are positive for oncogenic forms of the human papillomavirus (HPV), particularly 16 type.[2]

The most common high risk HPV types are HPV 16, HPV18, HPV33 and HPV35. These types are estimated to cause about 5% of the cancer burden worldwide, which include 99% of cervical cancer, 25%-60% of head neck cancer 70% of vaginal cancer, 88% of anal cancer, 43%valvar, 50%penile cancers.[5]

HPV infect the basal layer of epithelial cells through break in epithelial surface and maintained in the nuclei of infected basal cells. As the basal cell divides into squamous epithelial cells, HPV - DNA replicates and attains a high copy number. High risk HPVs produces 2 oncoproteins E6 and E7 that affect the apoptosis and cell cycle respectively.[6]They also divided according to their risk of cancer formation in high risk and low risk classes, with high risk classes having a high association with cancer formation. [7]

Normally HPV infections are not associated with malignant transformation since the majority of HPV infections are spontaneously cleared and most patients show and effective immune response against subsequent HPV infection.[5]

The high risk HPV was found to have potential to transform the normal cell to the malignant cell particularly in the oropharyngeal carcinoma.[8] HPV-positive oropharyngeal cancers can be distinguished from HPV negative tumors as a separate tumor entity in terms of etiology and clinical presentation.

HPV is detected by PCR & Real-Time PCR in tumour biopsy specimens. In a study done by Day P J et al. genotyping for high-risk types 16/ 18 was conducted by type specific PCR and p16 expression was assessed by immunohistochemistry. They concluded PCR is a selective target amplification assay capable of exponential and reproducible increase in the HPV sequences present in biological specimens. [9]

Recently studies suggest that HPV is a better prognostic marker than tumor stage in HNSCC, especially for oropharyngeal cancer, which spurs clinically interest on HPV testing. It is recommended that HPV testing should be used as a routine test in HNSCC management because it not only provides prognostic information, but also offers individualized treatment. [10]

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The aims of our study was to find the incidence of HPV in various subsites of squamous cell carcinoma of Head & Neck cancers and to compare the risk profile and clinical presentation of HPV positive and HPV negative patients.

2. Material and Methods

It is a prospective study, carried out in the department of ENT, Head & Neck Surgery and in The Central research laboratory from Dec 2015 to May 2017 at SRMS Institute of Medical Sciences, Bareilly, India, after approval by the research/ethics committee. The study group (SG) included all the patients who had been diagnosed as having squamous cell carcinoma of Head & Neck region. All the patients underwent detailed ENT history & examination. Radiological investigations in form of USG, CT scan, MRI were carried as per relevance on case to case basis.

In the patients suspected of having oral cancers multiple biopsies were taken by using a punch biopsy forceps. Fiber optic laryngoscope was used for oropharyngeal & laryngeal cancers for examination and biopsy.

One biopsy sample was sent for histopathology, the second biopsy sample was preserved in NET(NaCl, EDTA, Tris) buffer at -20^{o}C temperature till biopsy report arrives. The patients who were confirmed of having squamous cell carcinoma were examined in detail for risk factors & clinical profile. Their second preserved samples were sent for HPV. All the HPV samples were processed in Real Time - PCR in The Central research Laboratory of the institute.

The final data which was collected was of total 60 patients & was analysed on the basis of following parameters :

- Age & Sex distribution
- Duration of onset of symptoms
- Different habits - alcohol intake, Smoking habit, Tobacco intake
  Abnormal sexual behaviour
- Distribution of cancer at various subsites in Head & Neck region
- T staging of cancer
- Nodal status
- Stage of cancer
- Histopathological variant
- HPV 16 status

Entire study group was divided on the basis of HPV positive & HPV negative status & were compared on the above parameters. Statistical analysis was done by using SPSS 16.0.version.we used chi square test and concluded the results on the basis of p value. p value less than 0.05 was considered as significant. Analysis was done to compare risk profile & clinical presentation of both the groups & results were documented

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3. Results

Table 1: Comparison of Risk Profile and Clinical Presentation of HPV positive & HPV Negative Head and Neck Cancer

VARIABLE	ALL PATIENTS N=60 (%)	HPV POSITIVE N=9 (%)	HPV NEGATIVE N=51 (%)	p-VALUE
SEX				
Male	47(78.33)	8(88.89)	39(76.47)	0.404
Female	13(21.67)	1(11.11)	12(23.53)	
Average Age	47.85	50.89	47.31	0.544
Age Range				
20-30 years	3(5)	0(0)	3(5.88)	0.92
30-39 years	13(21.67)	2(22.22)	11(21.67)	
40-49years	10(16.67)	1(11.11)	9(17.65)	
50-59 years	22(36.67)	4(44.45)	18(35.29)	
60-69 years	11(18.33)	1(11.11)	10(19.60)	
70-79 years	1(1.67)	1(11.11)	0(0.0)	
HABIT				
Oral Tobacco	43(71.67)	**YES**	**YES**	0.689
Alcohol	21(35)	**NO**	**NO**	0.3
Smoking	30(50)	6(67.67)	37(72.54)	0.672
Abnormal Sexual	8(13.33)	3(33.33)	14(27.45)	0.00001
Behaviour		2(22.22)	19(37.25)	
		7(77.78)	32(62.75)	
DURATION OF		4(44.44)	26(50.98)	
SYMPTOMS	17(28.33)	5(55.56)	25(49.02)	
< 6 Weeks	19(31.67)	8 (88.89)	0 (0.0)	0.03
6 – 12 Weeks	11(18.33)	1(11.11)	0(0.0)	
12 – 18 Weeks	3(05)			
18-24 Weeks	10(16.67)			
>24 Weeks				
SUBSITE	39(65)	6(66.67)	11(21.67)	0.24
Oral Cavity	13(21.67)	2(22.22)	17(31.33)	
Oropharynx	6(10)	0(0.0)	11(21.67)	
Larynx	2(3.33)	0(0.0)	3(5.89)	
Nose & PNS		1(11.11)	9(17.65)	
'T' STAGING				0.07

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T1	33(55)	4(44.44)	35(68.63)	
T2	19(31.67)	2(22.22)	11(21.67)	
T3	6(10)	2(22.22)	4(7.84)	
T4	2(3.33)	1(11.11)	1(1.96)	
LYMPH NODE				0.48
N0	37(61.67)	4(44.44)	29(56.86)	
N1	19(31.67)	4(44.44)	15(29.41)	
N2	4(6.66)	1(11.12)	5(9.80)	
N3	0(0)	0(0.0)	2(3.92)	
STAGE				
Stage I	37(61.67)	7(77.78)	30(58.83)	0.771
Stage II	22(36.67)	2(22.22)	17(31.33)	
Stage III	8(13.33)	0(0.0)	4(7.84)	
Stage IV	2(3.33)	0(0)	0(0)	
HISTOPATHOLOGICAL TYPE				0.45
Well Differentiated	28(46.67)	4(44.44)	33(64.70)	
Moderately Differentiated	24(40.0)	3(33.33)	19(33.25)	
Poorly Differentiated	8(13.33)	2(22.23)	6(11.76)	
		0(0)	2(3.92)	
		6(66.67)	22(43.13)	
		2(22.22)	22(43.13)	
		1(11.11)	7(13.74)	

Age & Sex Distribution

The difference in mean age of HPV positive and HPV negative patient was not statistically significant. Though HPV positive patients were older (50.89yrs) than the HPV negative (47.31).Overall male female ratio was 3.6 : 1. In HPV positive patients it was 8 : 1 and in HPV negative patients it was found to be 3.2 : 1. Though HPV positive patients had a higher male female ratio, but no statistical significance was found.

Habit

Tobacco addiction was the most common habit in our series. Out of 60 cases 43(71.67%) had tobacco intake, 30(50%) were smokers, 21(35%) were regular consumers of alcohol and 8 (13.33%) cases have abnormal sexual behaviour. Out of 9 HPV 16 positive cases , tobacco intake was present in 6(67.67%) cases, alcohol consumption was present in 2(22.22%) cases , history of smoking was present among 4(44.44%) cases and abnormal sexual behaviour were present in 8(88.89%). Oral tobacco, Alcohol & smoking were not significantly associated

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with the HPV positive patients , whereas the association with abnormal sex behaviour was highly significant (p-value= 0.689 ,0.3 ,0.672 and 0.00001 respectively).

Duration of symptoms

Duration of symptoms at the time of presentation was < 6 weeks in majority of HPV positive patients. HPV positive patients presented earlier with symptoms(<6weeks) on comparison with HPV negative patients(between 6-12 weeks), which was statistically significant (p-value=0.0346) .

Subsites

On studying the distribution of Head and Neck squamous cell carcinoma in our series, it was observed that overall 39(65%) originated in the oral cavity out of which 4 were positive. Overall 13 cases of oropharyngeal cancer were observed, out of which 2 were HPV positive. Similarly out of total 6 cases of laryngeal cancer,2 were HPV positive. There was no predilection for specific site in HPV positive patients.

'T" Staging

Majority of the patients in our series presented as stage T1 and T2 (55.0% & 31.67% respectively). Maximum HPV positive as well as HPV negative patients also belonged to T1 & T2 Stage.

Lymph Node

Neck was clinically assessed for lymph nodes status. HPV positive head and neck cancers were more likely (77.7 %) present as N0 as compared to HPV negative(58.8 %). Only 2 (22.2%) HPV positive patients were N1 and none had N2 and N3 nodes. Nodal status is not show any statistical significance.

Stage and Histopathological Variant

Clinical staging was done and compared with the HPV status. Majority of the patients in both HPV positive and HPV negative study group presented as Stage I. We were not able to show any significant association with HPV positivity and staging. Similarly no correlation was observed with the degree of differentiation.

4. Discussion

Head and neck cancer (HNC) is a heterogeneous group of tumours characterized by a common anatomic origin, and most such tumours develop from within the mucosa and are classified as head and neck squamous cell carcinomas. The increasing epidemiological role of HPV & its value as a prognostic marker in Head & neck oncology has stimulated a growing number of studies in the past 10 years .[11]

HPV in Oral squamous cell carcinoma

Oral squamous cell carcinoma associated HPV 16 positive cases accounted for 10.29% (4 out of 39) in our study. To find prevalence of HPV in oral cavity cancers similar studies were done by Sritippho et al.(2015) and Tsimplaki et al.(2014) and they observed that HPV 16 was associated with OSCC with a prevalence of 20-25% and 11.3% respectively.[12,13] In contrast, because of usage of commercial PCR kits for the detection of HPV or genotyping DNA chips or reverse line blot hybridization and P16 immunohistochemistry, rather, much higher frequency of HPV DNA (74%) was observed in a study done by Seraj et al. [14]

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HPV in Oropharyngeal squamous cell carcinoma

HPV causes an estimated 30,000 oropharyngeal cancers worldwide each year. HPV is detected in 25% of all HNSCC, and the majority of these HPV associated HNSCC are oropharyngeal (tonsillar and base of tongue) squamous cell cancers.[15]

Worldwide in different population different incidences has been observed. We observed that in oropharyngeal cancers only 2 cases were positive out of 13(15%).In Germany and Switzerland authors have reported prevalence of 12% & 14% respectively .[16,17] On the contrary ,Black population showed higher prevalence(58.6%) in a study done by Zelvallos et al. They concluded that it might be due to the difference in HPV detection methods among their studies, which used type specific PCR, which is a highly sensitivity test and is more susceptible to misclassification and therefore can result in overestimation of HPV. [18]

HPV in Laryngeal squamous cell carcinoma

2 out of 6 (33%) laryngeal squamous cell carcinoma patients were HPV 16 positive. In Greek population of laryngeal squamous cell carcinoma detection of HPV DNA was seen in 18.5% patients.[19]

Association of HPV with Age

In our study group we found an overall bimodal age variation to be present with a peak at the fourth & sixth decade of life. Gillison et al. observed a first peak among patients aged 30-34 (7.3%) & second peak among patients aged between 60-64 (11.4%).[20]

HPV positive tumours have been found more common in younger age group.[21],but no significant association was seen between the age distribution & HPV positive tumours in our group. This was compared to a study done by Silva et al. Which also reported no association of HPV positivity with age. [22]

Mean age of HPV positive patients was found to be 50.8 yrs (>50yrs),whereas for HPV negative patients it was 47.3yrs (.50yrs) which was compared with study done by koppikar et al, where mean age group of patients was 51 yrs. [23]

Association of HPV with Sex distribution

We found a male predominance in our study group which was compared to another study done by Gillison et al and a study done by Singh et. al. They concluded an overall higher prevalence among men. This might be due to the fact that HNSCC is more common in males compared to females because of the presence of traditional risk factors like oral tobacco use. [20,24] On the other hand, in a study done by Mellin et al. it was observed that a significant association between the female gender & increased risk of HPV positive tumours specifically in oropharyngeal region was prevalent. [25]

Association of HPV with Habits

In General Head And neck cancers are highly associated with oral tobacco, smoking or alcohol intake but HPV Positivity in Head and neck squamous cell carcinoma showed no association with oral tobacco, smoking or alcohol in our study. Applebaum et al & Lindel et al. studied about these association ,where they also found alcohol & tobacco did not further increases risk of HPV 16 associated Head & Neck cancers. [26,17] Whereas a study done by Singh et al. showed an association between tobacco & HPV positive cancers, which they said was due to a high exposure of Indian population being exposed to oral tobacco.[24] Moreover

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we did not analyze the dose specific amount of alcohol, smoking or oral tobacco. If done, it could have given a better statistical correlation

HPV has been found to be highly associated with cervical cancers and abnormal sexual behaviour as a major cause of it. Hong et.al. found HPV to be common among young patients practicing oral sex more than 3 lifetime sexual partners.[21] We observed a significant association between abnormal sexual behaviour and HPV as well. We had 8 out of 9 patients showing abnormal sexual behaviour in the HPV positive tumour group, which was comparable with study of GIllison et al. and Kreimer et al. it was suggested that oral infection with HPV was predominantly sexually transmitted. [24,27]

On the basis of our results, we support the recent studies with the view, that etiology of HPV related Head & Neck squamous cell carcinoma is distinct from HNSCC associated with oral tobacco, smoking or alcohol.

Association of HPV with duration of symptoms

In a study done by Grosky et al and Pitcher & Martin reported patients of HPV positive OPSCC presented with early symptoms like neck mass.[28,29] We observed that majority of patients 66.6% in our study also presented with symptoms in < 6 weeks ,commonly as an ulceroproliferative growth.

HPV association with 'T" stage, N status, Stage of cancer and Histopathological variants

In our study group we did not find any association between HPV positivity and T staging, nodal status or stage at presentation. Whereas it was observed by Worsham et al that HPV positive head & neck cancers were more likely to be detected as late stage cancers. [31] Hong et al observed that HPV positivity was associated with lower T staging & higher N staging. [31] Gillison et al reported that poorly differentiated carcinomas are more likely to be HPV positive[20]. But again we observed no association was present between histopathological differentiate & HPV positivity of cancer.

5. Conclusion

It is evident in our study that the only risk factor significantly associated with HPV positive Head and neck cancer present was abnormal sexual behaviour and majority of them presented early. Also we concluded that:

1 Study done on comparison between risk factors & clinical presentation of HPV positive & HPV negative, Head & Neck squamous cell cancers, Lateral Border of tongue was the most common subsite of Head & Neck cancer in our region.

2.There was no association present between age & sex distribution with HPV positivity.

3.Oral tobacco, smoking & alcohol were not associated with HPV positive squamous cell carcinoma whereas abnormal sexual behaviour was highly associated with HPV positive cancers.

4.Human papilloma virus (HPV16) was significantly associated with squamous cell carcinoma of Head & Neck, especially with the oral cavity..

5.HPV positive Head & Neck squamous cell cancers had early onset of symptoms.

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6.There was no association present between T stage, Nodal status & staging of cancer with HPV positivity. HPV positive Head & Neck squamous cell cancers showed no association with different histopathological variants.

The potential limitations of this study that relatively small number of patients were detected as HPV positive. Thus for a definitive association of T staging, nodal status & staging of cancer with HPV positivity a large sample size was required.

Further studies are required to define treatment protocol and prognosticate the HPV positive Head & Neck squamous cell carcinoma, those may be suitable in Indian settings.

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