Original Article

DOI: 10.7860/JCDR/2020/42660.13629

Pathology Section

Evaluation of High Risk Pathological Features with Regards to HPV Status in Squamous Cell Carcinoma of Head and Neck

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ABSTRACT

Introduction: Positivity for Human Papilloma Virus (HPV) is an important prognostic factor and is associated with a favourable outcome in Head and Neck Squamous Cell Carcinoma (HNSCC). Patients with HPV positive tumours have a better response rate after chemo-radiotherapy. These patients have improved overall survival rates, a low risk of progression and death from any cause as in comparision to those with HPV negative tumours. There are few Indian studies related to HPV status in head and neck cancer patients, and none of them have been reported from Gujarat.

Aim: To study the incidence of HPV and to evaluate its histopathological features in HNSCC patients who have been treated with an intention to cure at Manibhai Shivabhai Patel Cancer Centre, Shree Krishna Hospital, Karamsad, Gujarat, India.

Materials and Methods: This was a retrospective study of 100 cases who underwent treatment for HNSCC at our cancer centre from January 2014 to January 2016. Details of clinical history and histopathological findings were recorded. Representative sections block from the tumour were sent to Gene Lab, Surat for HPV DNA PCR study. Results were analysed statistically by using STATA 14 software for Windows 7 and Microsoft Excel 2007.

Results: Out of 100 cases 11 cases (11%) were positive for HPV. Of these 6 patients (54.54%) were in age group 30-49 years, 10 patients (90.90%) were male, 10 patients (90.90%) had a habit of tobacco chewing. Out of 11 tumours, 7 (63.63%) were ulceroproliferative on gross appearance, 3 (27.27%) involved the tongue and 3 (27.27%) involved the buccal mucosa, 6 (54.54%) were well differentiated tumours, 4 (39.08%) showed lymph node metastasis, 4 (36.36%) were stage IV disease.

Conclusion: HPV is an aetiological agent in development of HNSCC. HPV positive HNSCC occur in middle aged men with a habit of tobacco chewing, early T category and N category as compared to HPV negative HNSCC. HPV positive SCC were predominately keratinising SCC and have marginally better prognosis, survival and response to treatment.

Keywords: Keratinising squamous cell carcinoma, Metastasis, Ulceroproliferative

INTRODUCTION

Squamous Cell Carcinoma (SCC) is the most common malignancy of head and neck and approximately 90% of all the tumours arise from this anatomic structure [1,2]. In the past decade, there has been a sharp increase in the incidence of HPV positive HNSCC particularly oropharyngeal cancers [3]. The prevalence of HPV positivity in tonsil and oropharyngeal cancer is higher than that in laryngeal or oral cavity cancers [4]. The increase in incidence of HPV-related Oropharyngeal Squamous Cell Carcinoma (OPSCC) is well documented in North America as well as in Europe and Australia [5,6], but it was not as much well established in South America, Asia, and Africa [7]. The percentage of OPSCC cases that were HPV positive increased from 16.3% in the period 1984-1989 to over 70% in 2000-2004 in the United States [5,8]. WHO has published the data of lip and oral cavity cancer in India in GLOBOCON 2016. According to the published data, the incidence was decreased from 7.2% to 6.4% fron 1990 to 2016 in India [9,10]. HPV-16 is the commonest subtype in all types of HPV positive cancers and HPV-18 is the next most common subtype [4,11].

If HPV positive HNSCC is treated with only surgery or chemotherapy or radiotherapy, it has shown good prognosis [12,13]. HPV positive status detection is useful in the planning and customising patient treatment regimes. HPV+ oropharyngeal cancer patients have a number of favourable demographic features as compared to HPV negative orpharyngeal cancer patients [14], HPV-related OPSCC is extremely sensitive to radiation exposure and patients generally have good prognosis and long-term survivors [15].

Very few studies are available from India related to HPV status in head and neck cancer patients and none have been conducted in Gujarat [16]. Due to high incidence of HNSCC especially OPSCC in Gujarat, this study was carried out to find out the incidence of HPV infection, to assess the specific strain of HPV [17] and to study clinical and histopathological variables as well as to assess outcomes in patients with HPV positive cancers who were treated with a curative intent at our cancer centre.

MATERIALS AND METHODS

The present study was a retrospective study of incidence and histopathological features of the HNSCC as well as to find out incidence of specific strain of HPV in reported cases. All the patients, who had undergone treatment (surgery or chemotherapy or radiotherapy) at Manibhai Shivabhai Patel Cancer Centre, Shree Krishna Hospital or any other hospital as well as traceable telephonically and were reviewed or reported at the Surgical Pathology Section of the Central Diagnostic Laboratory, Shree Krishna Hospital, Karamsad, Gujarat, India from January 2014 to January 2016. The study was ethically approved by Institutional Ethical Committee with ref no. (IEC/HMPCMCE/65/ Faculty/4/85/16). Approved study was used by medical oncologist and oncosurgeon for pre and postoperative counselling, instructions and follow-up [18].

Inclusion Criteria: First 100 cases of head and neck excisions or biopsy of patients with SCC who have been reviewed or reported, treated and traceable for follow-up during study period were selected for study.

Exclusion Criteria: Patients who did not fulfilled inclusion criteria and had undergone treatment but not traceable for follow-up were excluded from study.

Surgical Pathology

Following the receipt of surgical specimens in 10% formalin at the Surgical Pathology laboratory, patient identification and specimen verification were done. The small biopsy specimen were fixed for 4-5 hours and large biopsy were fixed for 12-14 hours in 10% neutral buffered formalin. Additional cuts were made depending on the size of the specimen. Detailed gross examination with cut surface appearance was recorded. Representative sections of tumour were submitted for paraffin block prepration and histopathological examination. The blocks were sectioned and stained with haemotoxylin and eosin stain. The histopathology requisition forms submitted along with the specimen were reviewed for intra-operative frozen section findings and the same were incorporated in the final histopathology report. Surgical pathology records pertaining to patient's clinical details were retrieved from the laboratory information system. In case of any inadequacy in the history, the information was obtained from the patients' medical records. In final report, details of sections submitted, histopathological findings including microscopy and macroscopy were included. If any additional findings were found, it was also mentioned.

Multiple histopathological characteristics were analysed microscopically, i.e., pattern of growth of tumour, tumour grade [19], stromal response, the degree of inflammatory response [20], lymphatic and vascular embolisation as well as for perineural invasion [21]. Histopathological staging of HNSCC patient was done according to UICC 7th AJCC staging [5]. Formalin fixed, paraffin embedded tissue was used for HPV. Representative blocks of the tumour were sent to Gene Lab, Surat for HPV DNA.

In Gene Lab, HPV detection was done by Polymerase Chain Reaction (PCR) and its genotyping by nucleotide sequencing.

Procedure

1. **DNA isolation:** DNA was extracted from paraffin block tissue by employing mini elute column and buffers supplied with the kit (Quick-DNA™ FFPE Kit, catalog no. D3067; Zymo Research Corp.) (Nanodrop Technologies Inc., Wilmington, DE). Quantity and purity of extracted DNA was checked by spectrophotometry (Nanodrop®) and DNA was stored at -20°C until PCR was performed.
2. **Nested PCR based detection of HPV:** A nested PCR was performed using MY09/11(L1 primer) as outer primers and GP5+/6+(L1 Primer) as inner primers. These L1 primer pairs amplify a 450 bp (MY09/11) and 140 bp (GP5+/6+) region of the HPV L1 gene. PCR reactions were performed according to the manufacturers' instructions using 5 µL of extracted DNA. A brief master mix containing 2U/µL Taq polymerase, 200nM of dNTPs, buffer with 2.5mM $MgCl_2$ and 200nM of each forward (PGMY09) and reverse (PGMY11) primers were prepared. We have used both forward as well as reverse primers for retrieving different HPV types like, HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68.
   - 2.1 **First and second round PCR:** The reaction mix for the first cycle of PCR was subjected to 35 cycles of amplification in the Thermal Cycle (Bio-Rad, Hercules, CA, USA), 1 minute denaturation at 94°C, 30 second annealing at 50°C and 30 second elongation at 72°C, with a final elongation step extended to 7 minute at 72°C for two times. To confirm absence of any such substance within the extracted DNA after second cycle, an inhibition control PCR reaction was carried out for every clinical sample with same method of nested PCR as mentioned above.
   - 2.2 **Agarose gel electrophoresis:** PCR products, which was generated from second round of PCR were run on a 2% agarose gel (Promega Corporation, Madison, USA), then it was stained with ethidium bromide (0.5 µg/ml), after that it was visualised under a UV source (260 nm). It was followed by the documentation using an automated gel documentation system.
   - 2.3 **HPV genotyping:** For the sample in which HPV DNA was detected, PCR products generated from nested PCR were purified, the purified PCR amplicon was then subjected to the double strand DNA sequencing using two pairs of sequencing reaction which were carried out using the Big Dye Terminator v 3.1 cycle sequencing kit (Applied Biosystems, USA) as per manufacturer's instructions. The sequence data generated was compared with reference genotypes of HPV using multiple sequence alignment tools and genotype was assigned to the clinical samples based on the most nearest sequence similarity with the reference genotype. Positive result band on agarose gel electrophoresis means one of the HPV genotype present i.e., HPV 6, 11, 16, 18, 31, 33, 45, and 58 [Table/Fig-1].

Size of the PCR amplicon (bp)	Interpretation
100	Detected: Successful extraction of DNA from the sample and absence of any PCR inhibitory molecules within the extracted DNA.
	Not Detected: DNA extraction failed or presence of some PCR inhibitory molecules within the extracted DNA sample. Repeat the entire experiment starting from the clinical sample.
144	Detected: HPV DNA present in the clinical sample.
	Not detected: HPV DNA present in the clinical sample but not extracted due to some inhibitory molecules.
450	Detected: Successful extraction of DNA from the sample and absence of any PCR inhibitory molecules within the extracted DNA.

[Table/Fig-1]: Showed interpretation of test results of HPV DNA PCR at different bps.

Quality of Life (QoL) Analysis

Outcome was assessed using the National Comprehensive Cancer Network/Functional Assesement of Cancer Therapy (NCCN FACT) questionnaire for head and neck cancer patients. Counselling of all 100 patients and their relatives was done, according to NCCN guideline for post-treatment care, diet and follow-up [22]. In all these patients, follow-up was done by telephonic conversation to assess outcome and for reminder of the advice given to them for care and diet during initial counselling.

STATISTICAL ANALYSIS

Statistical analysis was performed using a software program, STATA 14 software for Windows 7 and Microsoft Excel 2007. The correlation between HPV and HNSCC and the various histological parameters studied was analysed by univariate and multivariate logistic regression analysis.

RESULTS

The present study was undertaken to evaluate the high risk pathological features with regards to HPV status in patients of HNSCC treated with curative intent at a rural based cancer centre in Gujarat, India.

Most of the cases of HNSCC were in the age groups between 40-60 years, but it had no relation with HPV positivity and it was not statistically significant [Table/Fig-2]. Out of 100 patients, in 11 (11%) tumour showed HPV positivity [Table/Fig-2]. To study prevalence of HPV status in HNSCC different parameters like, demographic data, behavioural status, clinical features and morphological features were studied [Table/Fig-2].

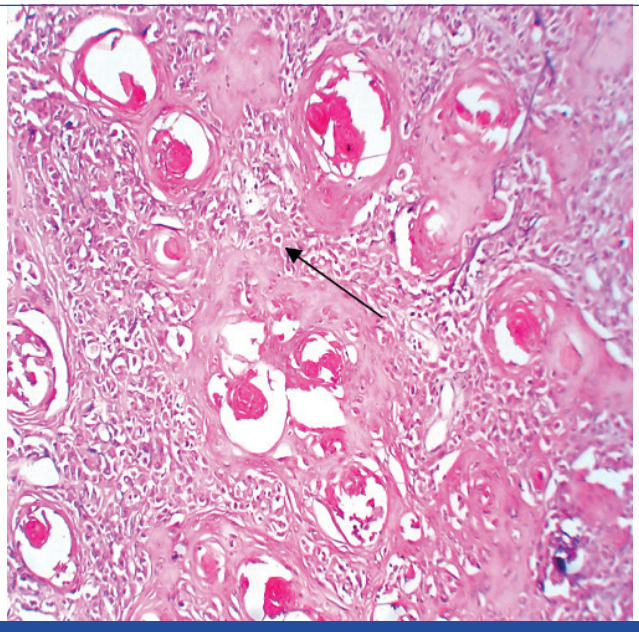
All 11 (11%) HPV positive patients were between age group of 30 years to 79 years.

There was no age specific positivity seen in studied subject and p-value for it was 0.123, which was also statistically non significant. From 11 HPV

	No. of patients	HPV status				p-value
		Positive		Negative		
		Total No. (11)	%	Total No. (89)	%	
Age (Years)						
20-29	04	00	0.00	04	4.49	0.123
30-39	26	03	27.27	23	25.84	
40-49	26	03	27.27	23	25.84	
50-59	21	02	18.18	19	21.34	
60-69	16	01	9.09	15	16.85	
70-79	05	02	18.18	03	3.37	
≥80	02	00	0.00	02	2.24	
Gender						
Male	86	10	90.90	76	85.39	0.523
Female	14	01	9.09	13	14.60	
Tobbaco use						
Yes	95	10	90.90	85	95.50	0.449
No	05	01	9.09	04	4.49	
Gross apparance						
Ulceroproliferative	48	07	63.63	41	46.06	0.345
Ulceroinfiltrative	52	04	36.36	48	53.93	
Tumour site						
Tongue	27	03	27.27	24	26.96	0.723
Buccal mucosa	35	03	27.27	32	35.95	
Alveolus	05	00	0.00	05	5.61	
Hard palate	01	00	0.00	01	1.12	
Soft palate	05	01	9.09	04	4.49	
Tonsil	03	01	9.09	02	2.24	
Cheek	01	00	0.00	01	1.12	
Pyriform fossa	03	00	0.00	03	3.34	
Gingiva and gingivobuccal sulcus	05	01	9.09	04	4.49	
Retromolar trigone	03	00	0.00	03	3.34	
Maxilla	03	00	0.00	03	3.34	
Floor of mouth	01	00	0.00	01	1.12	
Larynx	08	02	18.18	06	6.74	
Tumour size (cm)						
≤2 (T1)	27	03	27.27	24	26.96	0.837
2-4 (T2)	53	05	45.45	48	53.93	
>4 (T3)	20	03	27.27	17	19.10	
Grade						
Well differentiated	39	06	54.54	33	37.07	0.414
Moderate differentiated	42	03	27.27	41	46.06	
Poorly differentiated	19	02	18.18	17	19.10	
Nodal status						
Yes	34	04	36.36	30	34.48	0.552
No	66	07	63.63	59	52.87	
TNM stage						
I	21	03	27.27	19	21.34	0.776
II	18	02	18.18	16	17.97	
III	13	02	18.18	11	12.35	
IV	47	04	36.36	43	48.31	
Stromal response						
Inflammatory	96	11	100	85	95.50	0.623
Desmoplastic	04	00	0.00	04	4.49	
Myxoid	00	00	0.00	00	0.00	
Inflammatory response						
Mild	32	05	45.45	27	30.33	0.071
Moderate	52	05	45.45	47	52.80	
Severe	16	01	9.09	15	16.85	
Lymphovascular invasion						
Yes	03	00	0.00	03	3.37	NA
No	97	11	100	86	96.62	
Perineural invasion						
Yes	07	01	9.09	06	6.74	0.570
No	93	10	90.90	83	93.25	

[Table/Fig-2]: Showed frequency distribution of HPV prevalence status with clinical, histopathological, demographical and behavioural characteristics of patients by calculating p-value by STATA 14 software for Windows 7.

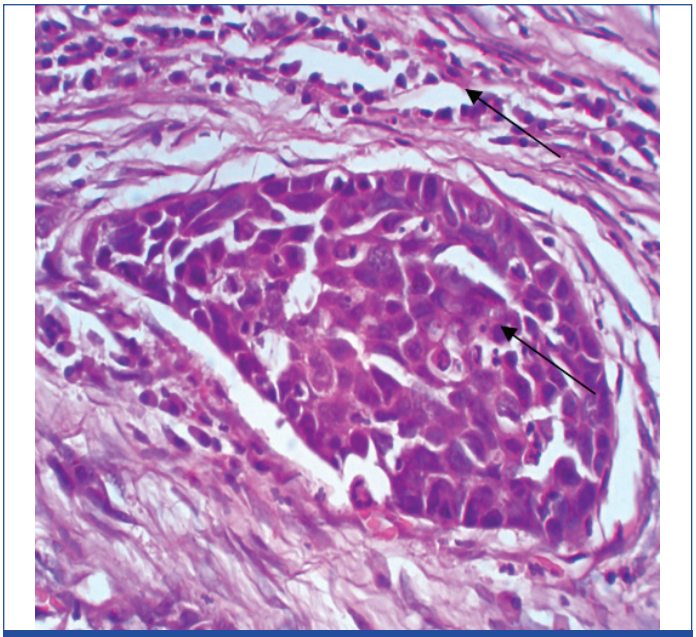
positive patients, 10 (10%) were male and all were tobacco chewers. The p-value for both were 0.523 and 0.449, respectively. Though, it was statistically non significant, tobacco chewing was proved as one of the risk factor. Different morphological parameters were studied in all 11 HPV positive patients like; gross appearance of tumour including site and size of the tumour, microscopic grading of tumour, stromal and inflammatory response, perineural invasion and lymphnode status. For all above parameters p-value was derived and it was >0.005. This suggests that, HPV positivity has no statistical significance regarding grading of tumour but majority of HPV positive tumours were well differentiated SCC, they showed keratin pearl formation and koilocytic changes on Haematoxylin and Eosin stained sections in microscopy [Table/Fig-3]. HPV positivity has no statistical significance with stromal response but in all positive tumour mild to moderate inflammatory stromal response was seen [Table/Fig-4,5]. Nodal metastasis and perineural invasion also have no statistical significance with HPV positivity but in all the cases no lymphnode metastasis or perineural invasion was seen. From above observation it was proved in present study that majority of HPV positive tumours were of well differentiated SCC with mild to moderate inflammatory stromal response, without perineural and lymphovascular embolisation. All were treated with surgery followed by chemotherapy. Lymphnode status was negative in all HPV positive so radiotherapy was not given to any patient. From continuous follow-up, it was also seen in present study that HPV positive patients have poor outcome, which was different from other published studies.



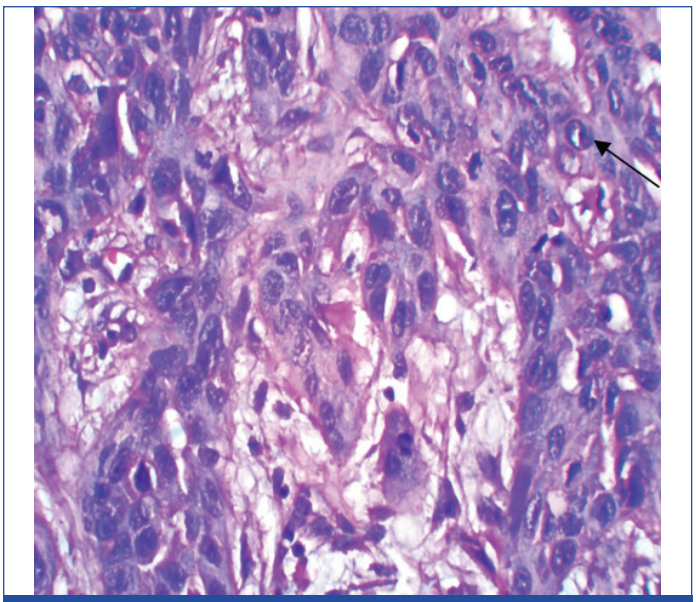
[Table/Fig-3]: Koilocytic changes and keratin pearls seen in HPV positive well differentiated squamous cell carcinoma.

DISCUSSION

Papilloma viruses are the members of the *Papovaviridae* family. HPV is a small, non-enveloped virus, with a diameter of 55 nm.



[Table/Fig-4]: Intercellular bridges and mild stromal response in HPV positive moderately differentiated squamous cell carcinoma.

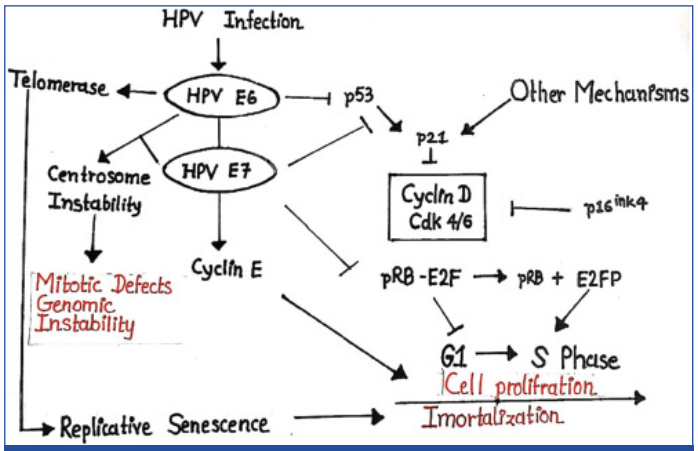


[Table/Fig-5]: Marked nuclear pleomorphism in HPV negative poorly differentiated squamous cell carcinoma.

It has an icosahedral capsid composed of 72 capsomers, which contain atleast two capsid proteins, L1 (major) and L2 (minor). Each capsomere is a pentamer of the major capsid protein and each virion capsid contains about 12 copies per virion of the minor capsid protein. The HPV genome is made up of a single molecule of double-stranded, circular DNA, which contains approximately 7.900 bp associated with histones of DNA [23].

There are more than 100 genotypes in HPV family, they are classified according to their ability to infect epithelial cells and cellular transformation [24]. Depending upon the ability of HPV to transform epithelial cells, it is divided into high risk and low risk types. Low risk types are associated with more of benign lesions like warts, while infections with high risk types progress to malignant lesions. Current test for HPV place their genotypes into low risk types (6, 11, 42, 43, 44) and high risk type (16, 18, 31, 33, 35, 39, 45, 51, 52, 58, 59, 68) [25].

HNSCC showed heterogeneity at histological, biological and clinical level and as a result, it is difficult to predict the outcome of this malignancy. Therefore, it is crucial to find molecular markers that define tumour subgroups with homogenous behaviour and HPV may represent one of them. In normal oral mucosal epithelium, p53 expression is limited to only basal cell layer [3]. In oral epithelial dysplasia, overexpression of mutated forms of p53 is considered as high risk factor for transformation to early stage OSCC. Amongst all, different studies showed that HPV16 is the most common genome in OSCC. Inactivation of the p16 gene is frequently identified during early carcinogenesis. However, normal oral mucosa and dysplasia showed negative result immunohistochemical staining with p16 [26]. It was evident from this study that, the expression of Rb protein was found to increase from normal through the various groups, precancerous to cancerous. The overexpression seen in oral cancer, with an increase in well-differentiated and moderately differentiated tumors suggest the possible role of Rb in differentiation [Table/Fig-6] [27].



[Table/Fig-6]: p53 HPV Rb pathway showing mechanism of HPV infection in carcinogenesis.

The prognosis and demographical characteristics of HNSCC have changed significantly over the last two decades [28-30]. The overall prevalence of HPV associated HNSCC worldwide varies from 0 to 30%. Region wise, Indian studies have reported a wide variation in prevalence of HPV associated HNSCC, ranging from 15 to 70.6% in Northeast, Eastern and Southern parts [17,31,32]. The present study found a positivity rate of 11% in the Anand district of Gujarat (Western India), which is low, as compared to other parts of India.

In the present study incidence of HPV positive HNSCC is comparable with study carried out by Portugal LG et al., (11%), Van houten VM et al., (14%), Lindel K et al., (14%), Dahlgren L et al., (9%) [33-36].

In study carried out by Brandwein-Gensler M et al., mean age was 55 years, Nasman A et al., reported mean age of 61 years, Dahlgren L et al., reported a mean age of 60 years, Attner P et al., reported mean age 62 years [20,30,36,37]. In this study, there was no age difference between patients with HPV positive and HPV negative HNSCC.

In the present study, in HPV positive HNSCC, gender difference was comparable with the study carried out by Mork J et al., Fakhry C et al., Hoffmann M et al,, and Chang JY et al., [38-41]. In above studies male and female incidence were 81% and 19%, 90% and 10%, 82% and 18%, respectively.

It was observed that tobacco consumption in any form is a risk factor and offenders have higher rate of HPV infection [Table/Fig-7] [35,39,42-45].

HPV Genotype

In the present study, out of 11 HPV positive HNSCC only 1(9.09%) was HPV-16, the other were type 6, 11, 42, 43, 44 with frequency of 2(18.18%) in all. In other studies majority of HPV positive HNSCC had HPV-16 type and other high risk types, which is a discordance with the present study [13,22,46-49].

Wide variations in size of tumour were noted in the present study. The spectrum of size distribution ranged from 0.5 cm to 8.0 cm while in the other studies- majority of the tumours were in T1 and T2 category [Table/Fig-8] [30,37].

Sl. No.	Authors	Tobbaco
		Yes
1	Klussman JP et al., [43]	96%
2	Lindel K et al., [35]	87%
3	Ritchie et al., [44]	90%
4	Smith et al., [45]	87%
5	Fakhry C et al., [39]	82%
6	Liang XH et al., [42]	87%
7	Present study	91%

[Table/Fig-7]: Showed tobacco is the major risk factor for HPV positive HNSCC by comparision with different studies [35,39,42-45].

Sl. No.	Authors	Size of tumour			
		≤2 cm (T1)	2-4 cm (T2)	≥4 cm (T3)	T4
1	Nasman A et al., [30]	25 (30%)	40 (48%)	13 (13%)	5 (5%)
2	Attner P et al., [37]	19 (27%)	23 (32%)	05 (7%)	24 (34%)
3	Present study	3 (27.27%)	5 (45.45%)	3 (27.27%)	0 (0.00%)

[Table/Fig-8]: Data of comparison of size distribution in HPV positive HNSCC between different studies with present study [30,37].

Some authors have combined two grades of differentiation. In the present study majority of the cases were well-differentiated, no combined grade of differentiation were used in this study [Table/Fig-9] [30,37,41,49].

Sl. No.	Authors	Grade of differentiation		
		Well differentiated	Moderately differentiated	Poorly differentiated
1	Chang JY et al., [41]	29 (57%)	16 (31%)	6 (12%)
2	Attner P et al., [37]	4 (6%)	18 (25%)	47 (66%)
3	Nassman A et al., [30]	1 (1%)	31 (37%)	50 (60%)
4	Kumar B et al., [49]	3 (100%)	0 (0.00%)	0 (0.00%)
5	Present study	6 (54.54%)	3 (27.27%)	2 (18.18%)

[Table/Fig-9]: Comparison of grade of differentiation in HPV positive HNSCC [30,37,41,49].

Some authors have combined two nodal stages for analysis. In the study by Chang JY et al., (82%) 42 cases were in N0 Stage and (18%) 9 in combined N1+N2+N3 Stage [42]. Fakhry C et al., quoted (34%) 13 in combined N0+N1 Stage and (66%) 25 cases in combined N2+N3 Stage [39]. Liang XH et al., (46%) 13 cases in in combined N0+N1 Stage and (54%) 15 cases in N2 Stage. Similarly few other authors had also evaluated the combined nodal status. [Table/Fig-10] [30,37,43,49].

Sl. No.	Authors	Lymph node status			
		N0	N1	N2	N3
1	Klussman JP et al., [43]	7 (30%)	5 (21%)	11 (49%)	2 (9%)
2	Attner P et al., [37]	15 (21%)	12 (17%)	40 (56%)	4 (6%)
3	Kumar B et al., [49]	0 (0.00%)	2 (67%)	1 (33%)	0 (0.00%)
4	Nassman A et al., [30]	6 (7%)	25 (30%)	49 (59%)	2 (2%)
5	Present study	7(63%)	2(18%)	2 (18%)	0 (0.00%)

[Table/Fig-10]: Comparison of lymph node metastasis in HPV positive HNSCC between different studies [30,37,43,49].

Outcome

Outcome was assessed by NCCN FACT questionnaires. It suggest that the overall outcome was poor as 50% of the patients enrolled in the study had passed away by December 2015. However, for patients with HPV positive HNSCC the outcome though numerically good was statistically not significant. It is difficult to assess the association of HPV positivity and prognosis especially since majority of the patients were at a higher stage of illness as compared to those reported by other authors. Overall, prognosis in HNSCC and HPV positive HNSCC in present study was poor which was different from other published studies.

Some research groups have advocated that HPV positive and negative HNSCC cases should not only be studied as clinicopathological entities but also on the basis of response to different treatment modalities [50-52]. Several studies have shown that patients with HPV positive HNSCC have a significantly improved overall and disease free survival compared to patients with HPV negative HNSCC patients [53]. HPV positive patients have better prognosis and responded well to therapy due to fewer or different genetic mutations identified in them [52], having higher radiosensitivity, probably due to intact apoptotic response to the therapy [17,54] absence of field cancerisation [55] and immunologic response playing a role in the improved response to chemotherapy and radiotherapy [55,56].

Limitation(s)

The limitations of this study are related to the fact that all the patients of Head and Neck Squamous Cell Cancers, reported during the study period could not be included due to lack of traceability of patients or due to death of the patient happen before the study was started.

CONCLUSION(S)

HPV positive HNSCC reported in majority of middle aged men with habit of tobacco chewing and were of early T and N category. There was moderate aggrement found between well differentiated and keratinising SCC with HPV positivity. Though HPV related head and neck cancers have better survival rate, there was no relation found between HPV positivity with outcome of treated patients. There is limited and incomplete data available regarding the better survival rate of HPV positive head and neck cancers. In future, further studies will be needed to establish the effectiveness of HPV vaccine in prevention of head and neck cancers like it is being used in prevention of anogenital cancers.

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PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Aug 23, 2019
- Manual Googling: Feb 12, 2020
- iThenticate Software: Mar 20, 2020 (19%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Aug 22, 2019**
Date of Peer Review: **Sep 22, 2019**
Date of Acceptance: **Feb 29, 2020**
Date of Publishing: **Apr 01, 2020**