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Original Research Article

Study of Human papillomavirus (HPV) - Related Oropharyngeal Squamous Cell Carcinoma as a distinct entity in Head and Neck Squamous Cell Carcinoma (HNSCC)

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Abstract

Background: Human papillomavirus (HPV) associated head and neck squamous cell carcinoma (HNSCC) is an entity with unique clinical and molecular characteristics. The detection of HPV is clinically important and for that several methods are currently available. Aim of the present study was to determine the histomorphological features of HPV-related oropharyngealsquamous cell carcinomas (OPSCC) and to detect HPV using p16 IHC staining and HPV DNA in situ hybridization/IHC.

Method: A total 17 cases of HNSCC were studied during a period from 1st January 2013 to 30th June 2014. Tumors with clinical and histopathological features of HPV related HNSCC were tested for HPV by p16 IHC staining and HPV DNA In situ hybridisation/IHC.

Results: Among 17 patients, 13(66.5%) had conventional SCC and 4(23.5%) patients had HPV-related OPSCC.Most cases of HPV-related OPSCC were in 6th decade (M: F= 1:1) while majority of conventional SCC were in 7th decades with male predominance (61.53%). Tonsil & palate (75%) was the commonest site of tumor in cases of HPV-related OPSCC while in conventional SCC, buccal mucosa, tongue, gingiva, pharynx, and larynx were the commonest site of tumor (92.30%). 2 cases of HPV-related OPSCC had history of tobacco addiction as compared to 8 in conventional SCC. In HPV-related OPSCC histopathological figures was non-keratinizing tumor with tumor infiltrating lymphocytes while the features of conventional SCC were of keratinizing SCC infiltrating in cords and nests with desmoplasia and surface dysplasia.

Conclusion: HPV related HNSCC has a distinct histomorphology and clinical presentation. p16 and ISH are useful HPV detection methods in fixed tissue.

Keywords: Human papillomavirus; Head and neck squamous cell carcinoma; Histomorphological; p16 IHC; HPV-DNA in situ hybridization/IHC.

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1. Introduction

Human papillomavirus (HPV) is non-enveloped icosahedral, circular, dsDNA virus that can infect cutaneous and mucosal epithelia. Approximately 200 different HPV genotypes have been identified responsible of a broad spectrum of the clinical profiles, from benign lesions to HPV-related carcinomas [1, 2]. HPV-related carcinogenesis has been attributed to a subset of head and neck cancers [3, 4]. Those cancers comprise squamous cell carcinoma of the head and neck (HNSCC), and primarily arise in the oropharynx (OPSCC) and especially in the tonsils [5, 6]. HPV-related HNSCCs have emerged as a form of HNSCC

with an epidemiologic, demographic, histopathologic and clinical profile deviating from profile of conventional non-HPV related HNSCC. Histologically, HPV-associated OPSCC exhibit nonkeratinizingbasaloid morphology; occur in younger age group with improved clinical outcome whereas HPV-negative OPSCC is classically keratinizing [7, 8]. HPV testing is recommended for prognostication and to resolve diagnostic dilemmas in patients with HNSCC.

Several methods for HPV detection are currently available including Polymerase chain reaction (PCR)-based techniques, DNA in situ hybridization (ISH), RNA ISH, and p16 immunohistochemistry (IHC) [9]. Currently, the guidelines for HPV detection in cervical carcinoma are available, while no clear consensus has not yet been reached on the gold standard for HPV testing in OPSCC. The current guidelines from the College of American Pathologists (CAP) as well as American Society of Clinical Oncology (ASCO) recommend testing for HPV in tumor samples of OPSCC using surrogate marker p16 IHC [10, 11].Performing subsequent confirmatory HPV-specific tests is at the discretion of the pathologist. Previously, studies have variably used HPV-specific tests such as HPV DNA–ISH, DNA polymerase chain reaction (PCR), mRNA RT PCR and mRNA ISH for viral oncoproteins E6 and E7, as well as IHC staining of HPV surrogate marker p16. The detection of HPV E6/E7 mRNA by PCR is considered the gold standard. However, the test cannot be performed directly on the readily available formalin-fixed paraffin-embedded (FFPE) specimens and requires cumbersome preparations of the tissue sample, making it impractical for use in most clinical context. A more recently available test, HPV E6/E7 mRNA ISH which can be performed on FFPE specimens has been validated against PCR, HPV–DNA ISH and p16 IHC and shown to have high concordance rate with p16 IHC in OPSCC [12]. This test, however, is also not widely available and hence, cannot be used for large multinational studies or routine clinical practice. For these reasons, p16 IHC and HPV–DNA ISH, which can be performed on readily available formalin-fixed, paraffin-embedded (FFPE) tissues samples and are the widely used testing modalities for HPV detection. The present study was undertaken to determine the histomorphological features of HPV related OPSCC and to detect HPV using p16 IHC staining and HPV DNA in situ hybridization/IHC.

2. Materials and Methods

A total 17 cases of squamous cell carcinoma of head and neck region were study during a period from 1st January 2013 to 30th June 2014. All cases were reviewed according to the histopathological classification. The histological features studied were surface dysplasia, growth pattern, desmoplasia, keratinization, tumor differentiation and presence of tumor-infiltrating lymphocytes. Tumors with clinical and histopathological features of HPV related HNSCC were tested for HPV by p16 IHC staining and HPV DNA In situ hybridisation/IHC

p16 immunohistochemistry

P16 immunoreactivity was evaluated using the CIN tec p16INK4a Histology Kit (Roche Diagnostics). A tumor has been considered positive in the case of strong nuclear and cytoplasm decoration in >50% of tumor cells. Immunohistochemistry has been performed on an automated immunostainer (BenchMark Ultra, Ventana Roche) according to the company's instructions.

HPV DNA In situ hybridisation/IHC

DNA-based ISH has been performed using both the Inform HPV III family probe (Ventana Roche), a cocktail of probes recognizing HPV types. It has been performed on an automated immunostainer (BenchMark Ultra, Ventana Roche) according to the company's instructions. For each case the percentage of pattern of infection (integrated versus episomal) has been recorded.

Descriptive statistics were used to summarize the data (Mean and range for age, and frequency and percent for categorical data).

3. Observations and Results

During the study period, a total of 17 cases of squamous cell carcinoma of head and neck region were enrolled in the study. Table 1 show the anatomical distribution of tumor. The most common site of tumor was tongue (23.52%) followed by tonsil and palate (17.64%) as well as larynx (17.64%).

Table 1: Showing Anatomic Distribution

Site of tumor	No. of patients	Percentage
Buccal mucosa	02	11.76
Tongue	04	23.52
Gingiva	02	11.76
Tonsil and palate	03	17.64
Pharynx	01	5.88
Larynx	03	17.64
Metastasis to node	02	11.76
Total	17	100

Out of 17 cases, 4 (23.5%) patients were diagnosed as HPV-related OPSCC.Case 1 and case 2- HPV DNA by CISH was positive while p16 was overexpressed. Case 3 – HPV DNA by CISH was equivocal while p16 was overexpressed whereas case 4- HPV DNA by IHC was negative while p16 was overexpressed (intensely positive), (Table 2 and figure 1).

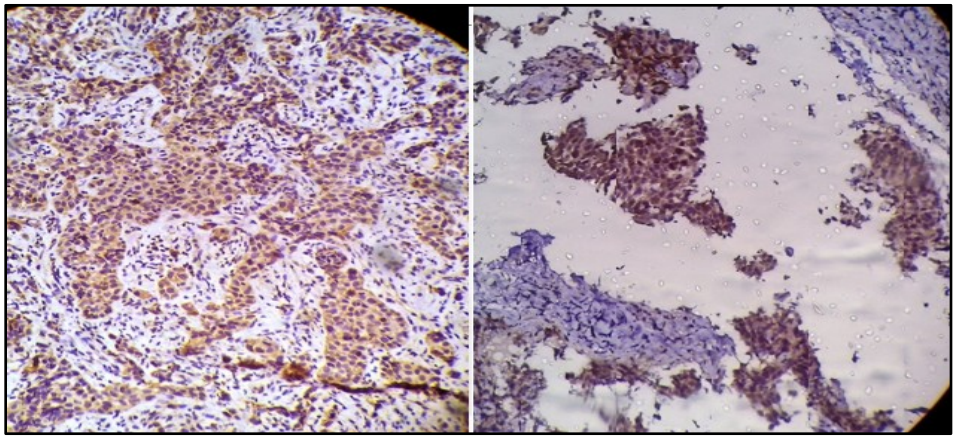
Table 2: HPV testing

HPV TESTING	HPV DNA (by CISH/IHC)	P16 (by IHC)
Case 1	+	+
Case 2	+	+
Case 3	+/-	+
Case 4	_	+

Abbreviations:

CISH- Chromogenic in situ hybridisation, IHC-Immunohistochemistry

Figure 1: p16 IHC- Tumor cells showing nuclear & cytoplasmic positivity (100x)

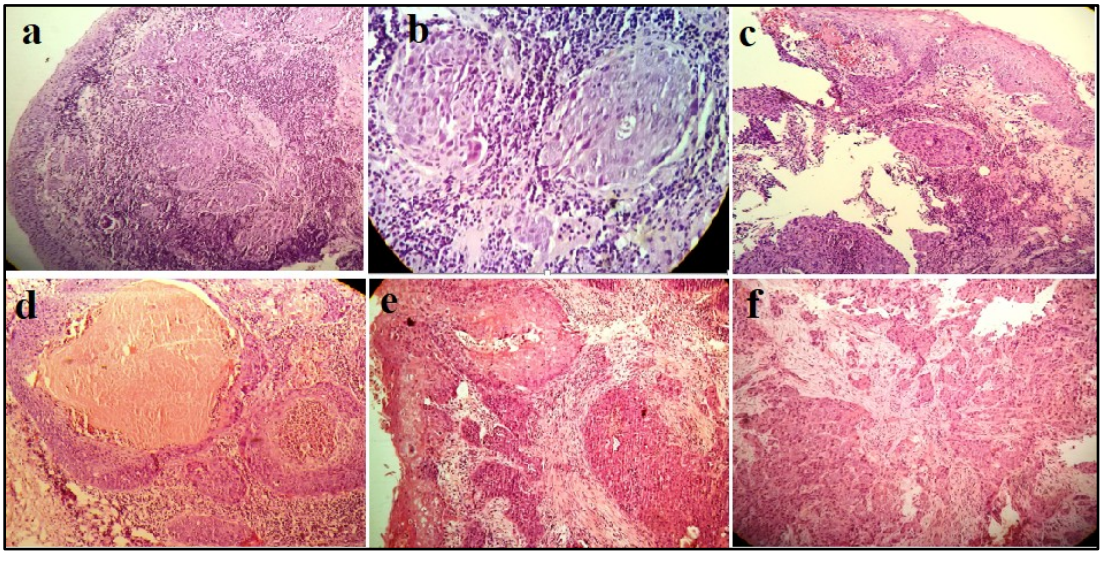


Among the 17 cases, 13 (66.5%) patients had conventional SCC and 4 (23.5%) patients were diagnosed as HPV-related OPSCC. Histomorphological features of conventional SCC and HPV-related OPSCC are depicted in table 3 and figure 2.

Table 3: Histomorphological Features

Histological features	Conventional SCC	HPV-related OPSCC
Origin	Surface epithelium	Reticulated epithelium
Growth pattern	Irregular cords and nests	Sheets and rounded lobules
Desmoplasia	Prominent	Often absent
Keratinization	Prominent	Minimal
Differentiation	Moderately differentiated	Basaloid
Tumor – infiltrating lymphocytes	Absent	Present
Surface dysplasia	Present	Present in two cases
Other morphologic variants	Basaloid SCC in larynx	-
Number of cases	13(66.5%)	04(23.5%)

Figure 2: Histopathology show- HPV-related OPSCC- a) sheets & lobules of tumor cells with basaloid morphology (100x); **b)** Non keratinizing tumor with tumor infiltrating lymphocytes (400x)**; Conventional SCC- c)** sheets & lobules of tumor cells with basaloid morphology (100x); **d)** Metastatic SCC with cystic degeneration (100x); **e)** Tumor(keratinizing SCC) infiltrating in sheets & cords with surface epithelium showing dysplasia (100x); **f)** Tumor infiltrating in cords with surrounding desmoplasia (100x)



Most cases of HPV related OPSCC were in 6th decade (M: F= 1:1) while most cases of conventional SCC were in 7th decades with male predominance (61.53%). Tonsil & palate (75%) was the most common site of tumor in cases of HPV related OPSCC while in conventional SCC, buccal mucosa, tongue, gingiva, pharynx, and larynx were the most common site of tumor (92.30%). 2 out of 4 cases of HPV related OPSCC had history of tobacco addiction compared to 8 out of 13 in conventional SCC as shown in table 4.

Table 4: Clinical Features

Clinical Features		Conventional SCC (n=13)	HPV-related OPSCC (n=4)
Age (in years)	Mean age (Range)	63 (51-81)	56 (48-72)
	5th decade	1 (7.69%)	1 (25%)
	6th decade	3 (23.07%)	2 (50%)
	7th decade	6 (46.15%)	-
	8th decade	2 (15.38%)	1 (25%)
	9th decade	1 (7.69%)	-
Sex	Males	08 (61.53%)	02 (50%)
	Females	05 (38.46%)	02 (50%)
	M:F ratio	1.6:1	1:1
Site	Buccal mucosa, Tongue, Gingiva, Pharynx, Larynx	12 (92.30%)	00 (0.0%)
	Lymph node metastasis	01 (7.69%)	01 (25%)
	Tonsil & palate	00 (0.0%)	03 (75%)
Chronic Tobacco addiction		8 (61.53%)	2 (50%)

4. Discussion

HPV-positive oropharyngeal squamous cell carcinoma represents a unique cancer type with distinct clinicopathologic features and favorable prognosis. Thus HPV-OPSCC is now a well-recognized tumoral entity in the field of head and neck oncology, with an increasing incidence worldwide [11, 13]. Today, 70% of OPSCC cases are HPV-associated. There is growing body of evidence that HPV positive OPSCC differs significantly from HPV negative OSCC in terms of risk-factor profiles, molecular characteristics, and clinical-pathologic features. Notably, patients with HPV-positive OSCC have a better prognosis compared to those with HPV-negative OSCC and most likely benefit from specific treatments [14, 15].

In the present study, out of 17 cases of head and neck SCCs, 4 were HPV related SCCs (23.5%) of all head and neck SCCs and 23% amongst oropharyngeal SCCs). In study by Kreimer *et al* in 2005, HPV infection was found in 25.9% of head and neck cancers and 35.6% of oropharyngeal squamous cell carcinomas.Most cases of HPV related OPSCC were in 6th decade followed by 5th and 8th decade with mean age of patients was 56 years while most cases of conventional SCC were in 7th decades followed by 6th and 8th decade with mean age of patients was 63 years. Gender distribution was equal in HPV related OPSCC group whereas male predominance (61.53%) was observed in conventional SCC. Tonsil & palate was the most common site of tumor in cases of HPV related OPSCC while in conventional SCC, buccal mucosa, tongue, gingiva, pharynx, and larynx were the most common site of tumor. 2 out of the 4 cases of HPV related OPSCC had history of tobacco addiction compared to 8 out of 13 in conventional SCC. Cohen and Psyrri [16] have also found that HPV-positive patients tend to be younger with less exposure to tobacco. Our findings of HPV related OPSCCs are as per Westra[17] showed a nonkeratinizing SCC with basaloid morphology; infiltrating in lobules with surrounding tumor infiltrating lymphocytes. However we found surface dysplasia in two case. This probably can be attributed to tobacco addiction

The features of conventional SCC were of a keratinizing SCC infiltrating in cords and nests with desmoplasia and surface dysplasia as described in literature.As per Westra [17], HPV testing is also employed to resolve diagnostic dilemmas and look for primary in neck metastasis with unknown primary. We had one case of SCC metastasis to neck node of unknown primary showing basaloid morphology with cystic change. When HPV testing was done in this case,it was positive and therefore it suggested a search for primary in oropharynx. FDG PET scan done subsequently showed accentuated uptake in in the soft palate which points to occult primary in oropharyngeal region.

One case of HPV-associated SCC presented with neck swelling. Excision biopsy showed SCC metastasis with cystic change. No primary was found on clinical examination. HPV testing was employed as it is a reliable predictor of oropharyngeal origin Both HPV DNA by CISH and p16 IHC were positive. Case 1 and case 2-HPV DNA by CISH was positive while p16 was overexpressed. As per Jordan *et al* [18], when combined ISH and p16 overexpression are considered together sensitivity was 86.1% while specificity was 97.3%. Case 3 –HPV DNA by CISH was equivocal while p16 was overexpressed. When either ISH or p16 are positive, sensitivity was 98.7% while specificity was 81.1%. Case 4-HPV DNA by IHC was negative while p16 was overexpressed (intensely positive). As per Lin and Prichard [19], a negative test by IHC does not rule out the presence of HPV as many HPV types are not detected on IHC. Accurately diagnosing HPV positivity in OPSCC is important as it confers better prognosis. Numerous de-intensification treatment strategies are under investigation for HPV-associated OPSCC that can potentially reduce treatment related toxicities and morbidities while maintaining similar progression-free survival and overall survival.

5. Conclusion

HPV related HNSCC has a distinct histomorphology and clinical presentation. They tend to

present at younger age,have a basaloid morphology and have predilection for tonsil and palate. p16 and ISH are useful HPV detection methods in fixed tissue. HPV testing can also be employed for indicating primary of oropharyngeal origin when metasatsishas characteristic morphology.

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