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Original Research Article

P16 expression and clinicopathological features of oral and oropharyngeal squamous cell carcinoma

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ABSTRACT

Background: There is an epidemiological shift in head and neck squamous cell carcinoma (HNSCC) attributable to HPV infection. HPV positive HNSCC has unique biology, risk factors, clinicopathological characteristics and outcome. There is a large variation in the published prevalence of HPV-related HNSCCs in India ranging from 7 to 78.7%. This study aims to find the P16 expression in the oral cavity and oropharyngeal SCC, thereby prevalence of HPV in our setting and to define the clinicopathological characteristics of HPV positive tumours in our setting.
Methods: 210 specimens of primary Oral squamous cell carcinoma (OSCC) and Oropharyngeal Squamous cell carcinoma (OPSCC) were included. Immunohistochemistry was done using monoclonal mouse p16 antibody. Clinical details of each case were collected. Analysis was done using SPSS software and the association of P16 and clinicopathological variables were calculated using Fishers exact test.
Results: P16 positive expression is observed only in 1/122 (0.82%) of OSCC and 8/88 (9%) of OPSCC. P16 positivity showed significant association with Grade of tumor (p= 0.008) and histological variant of SCC (p=0.00). 77.7% of P16 positive tumours are Grade 2 and 66.6% of Basaloid SCC was P16 positive. There is no significant association between p16 expression and other variables (subsite, age, gender, alcoholism, smoking, betel chewing and stage).
Conclusions: P16 positivity was higher in oropharyngeal than in oral cancer. However, the HPV positivity rates are lower than other parts of India.

Keywords: P16 immunohistochemistry, HPV, Oral Squamous cell carcinoma, Oropharyngeal squamous cell carcinoma

INTRODUCTION

Squamous cell carcinoma of head and neck is a major public health problem in developing countries.[1] Oral cavity and oropharynx squamous cell carcinoma constitute most of the head and neck cancers. Overall oral and oropharyngeal squamous cell carcinoma is the sixth most common cancer in the world. Incidence of oral cancer is found to be high in Southern Asia and age standardized incidence rates in India and Pakistan is more than 10 cases per 1,00,000 population.[2]

Smoking and alcohol act synergistically and are the two most important etiological factors responsible for about 75% of head and neck squamous cell carcinoma. Despite the decrease in the incidence of tobacco induced cancers in many countries, there is an increasing incidence of HNSCC, particularly in younger age group even in the absence of above risk factors. This shift in epidemiology has been attributed to Human Papilloma Virus (HPV) infection.[3] HPV was included as a carcinogenic agent in the head and neck squamous cell carcinoma (HNSCC) by International Agency for Research on Cancer (IARC) in

2007.[4] The majority of HPV-related squamous cell carcinoma arises in the oropharynx, particularly the palatine and lingual tonsils. In oral cavity, only a small proportion of cases appears to be caused by HPV.[5] HPV positive squamous cell carcinoma has unique biology, risk factor profile, clinicopathological characteristics and outcome. HPV positive OPSCC, although presents at a later clinical stage, is associated with significantly better survival outcome than HPV negative OPSCC.[6] Demonstration of P16 overexpression by immunohistochemistry is the surrogate marker of transcriptionally active HPV infection. According to the recent AJCC (8th edition) guidelines, P16 IHC should be done in all oropharyngeal cancers with a separate staging system for HPV positive OPSCC.[7]

In the large population based epidemiological study by Brandon et al., the incidence of HPV-positive OPSCC was 4.62 per 100,000 persons, 250% the incident rate of HPV-negative OPSCC in the United Staes.[8] Even though the literature on HPV-positive OPSCC cancers in India is limited, in the study published by Bahl et al in 2013, the reported prevalence of HPV in OPSCC was 22.5% in Northern India.[9] Murthy et al and Sannigrahi et al reported a similar prevalence of 20% and 15% respectively.[3,10]

Yete et al observed 50 studies all around the world and reported the average prevalence of HPV-positive oral cancer as 24.4%. However, the prevalence in India was 36.6% which is slightly higher than the global prevalence.[11] Balaram et al reported a high prevalence of 73.6% in the South India (1995)12, while prevalence in the North and Northeast ranged from 7 to 29% 15% in the western region, 33.6% in the Eastern region and 27.5% prevalence in Central India.[13-17] The published literature regarding prevalence of HPV in Kerala is limited, except for study by Balaram et al in 1995. Hence, this study aims to find the P16 positive expression in the oral cavity and oropharyngeal squamous cell carcinoma, thereby the prevalence of HPV infection in our setting and to define the clinicopathological characteristics of HPV positive tumours in our setting.

METHODS

This is a descriptive study conducted on 210 cases of primary oral or oropharyngeal squamous cell carcinoma which are histopathologically diagnosed in the Department of Pathology, Govt. Medical College, Kottayam during the study period of 18 months (January 2019 to June 2020). The study was approved by the Institutional Review Board of Government Medical College, Kottayam.

Proportion of oropharyngeal squamous cell carcinoma cases which showed p16 positivity in study conducted by Adilbay18 et al was 25.7%. Taking absolute precision as 6, Sample size was calculated with formula, N=Zα2pq/d2. Sample size was taken as 210. Continuous sampling was done. First 210 cases of Squamous cell carcinoma of oral cavity and oropharynx were included. Inadequate biopsy specimens for doing Immunohistochemistry, carcinoma infiltrating oral cavity or oropharynx from adjacent areas like nasal cavity, paranasal sinuses, nasopharynx, glottis, thyroid, salivary gland and metastasis to oral cavity or oropharynx were excluded.

All specimens with clinical diagnosis of Squamous cell carcinoma of oral cavity and oropharynx were fixed in 10% formalin. After 24-hour formalin fixation and processing, all specimens were embedded in paraffin. 4-micron thickness sections were made and stained with Haematoxylin and Eosin for routine histological examination. Carcinoma infiltrating oral cavity or oropharynx from adjacent areas like nasal cavity, paranasal sinuses, nasopharynx, glottis, thyroid, salivary gland are excluded.

Immunohistochemistry was performed using monoclonal mouse p16 antibody (Biogenex, Anti- P16(INK4), Clone G175-405, AM540-2M) on histopathologically diagnosed cases of oral cavity and oropharyngeal squamous cell carcinoma specimens and evaluated with a light microscope. A positive control of known p16-positive cervical squamous cell carcinoma was run with each batch of test slides. P16 expression is considered positive if there is strong and diffuse nuclear or nucleocytoplasmic staining present in greater than 70% of the malignant cells. All other staining patterns were considered negative.

Clinical details of each case were collected from the available records in the Medical Records Library and recorded as in Proforma. Clinicopathological variables studied include site – oral cavity/ oropharynx, subsites, age, Gender, Conventional/ Histological variants, Grade, Risk factors- alcoholism, smoking, betel nut chewing independently and stage of disease. Histopathologic grading was done according to World Health Organization criteria. Grade 1: Well differentiated, Grade 2: Moderately differentiated and Grade 3: Poorly differentiated

Analysis was done using Statistical package for social sciences (SPSS) software (version 26). Descriptive data was presented in the form of frequency and percentage and the association of P16 and clinicopathological variables were calculated using Fishers exact test. $P<0.05$ was considered statistically significant.

RESULTS

Among the study participants, 122 had Oral Squamous cell carcinoma (OSCC) and 88 had Oropharyngeal Squamous cell carcinoma (OPSCC). Within oral cavity, oral tongue was the most common site, followed by buccal mucosa. Base of tongue was the most common site in oropharynx, followed by soft palate. Maximum incidence was observed within the age group 60-69 years and mean age calculated was 60.4 years. Mean age of OSCC was 58.7 years and OPSCC was 62.8. Marked male predominance was observed, especially in oropharynx. Conventional Squamous cell carcinoma is the most encountered entity in this study.

Table 1: Demographic details of study population.

	Oral cavity		Oropharynx	
Total no of cases	122 (58.1%)		88 (41.9%)	
Subsite	Oral tongue	58 (48%)	Base of tongue	27 (31%)
	Buccal mucosa	36 (29%)	Soft palate	17 (19%)
	Floor of mouth	7 (6%)	Tonsil	18 (20%)
	Lower alveolus	6 (5%)	Posterior pharyngeal wall	13 (15%)
	Upper alveolus	4 (3%)	Anterior pillar of tonsil	6 (7%)
	Hard palate	6 (5%)	Valleculae	6 (7%)
	Retromolar trigone	5 (4%)	Uvula	1 (1%)
Age (in years)				
20-29	1 (0.8%)		0 (0%)	
30-39	7 (5.7%)		0 (0%)	
40-49	18 (14.7%)		6 (6.8%)	
50-59	38 (31.1%)		23 (26.1%)	
60-69	35 (28.7%)		39 (44.3%)	
70-79	18 (14.7%)		17 (19.4%)	
80-89	5 (4.1%)		3 (3.4%)	
Gender				
Male	97 (79.5 %)		86 (97.7 %)	
Female	25 (20.5 %)		2 (2.3 %)	
Classic /Variants				
SCC, NOS	117 (96 %)		82 (93 %)	
Verrucous carcinoma	3 (2.4 %)		6 (7 %)	
Acantholytic SCC	2 (1.6 %)		0 (0%)	
Basaloid SCC	0 (0%)		0 (0%)	
Grade				
Grade 1	79 (66.4 %)		31 (35.2 %)	
Grade 2	36 (30.2 %)		47 (53.5 %)	
Grade 3	4 (3.4 %)		10 (11.3 %)	
Risk Factors				
Alcoholic	82 (67.3%)		82 (93.1%)	
Non-Alcoholic	40 (32.7%)		6 (6.8%)	
Smoker	92 (75.4%)		84 (95.4%)	
Non- smoker	30 (24.6%)		4 (4.6%)	
Betel Nut chewer	69 (56.6%)		39 (44.3%)	
Non chewer	53 (43.4%)		49 (55.7%)	
Stage of tumour				
Stage I	29 (23.8%)		15 (17.0%)	
Stage II	33 (27.0%)		14 (15.9%)	
Stage III	35 (28.7%)		19 (21.6%)	
Stage IV	25 (20.4%)		40 (45.5%)	

Rare variants diagnosed include verrucous carcinoma and acantholytic squamous cell carcinoma in oral cavity and basaloid SCC in oropharynx. In OSCC, majority of cases are well differentiated Grade 1 tumour, while majority in OPSCC are grade 2 tumours. In both categories, the least are Grade 3 tumours, 3.4% and 11.3% respectively.

Habit of alcohol consumption was found in 67.2% of OSCC patients and 93% of OPSCC patients. 75.4% of patients with OSCC and 95.4% of patients with OPSCC are smokers. 56.5% of patients with OSCC have the habit of betel nut chewing while in the OPSCC, only 44.3% of patients are betel nut chewers. Patients with OSCC presented in almost equal proportion of Stage III, stage II and Stage I disease. In the oropharynx, most of the patients presented in Stage IV. (Table 1)

Table 2: Clinicopathological characteristics of P16 positive SCC in the study group.

Variable	Distribution	P16 positivity (n=9)	P value
Site	Oral cavity	11.1% (n=1)	0.004
	Oropharynx	88.8% (n=8)	
Subsite	Oral tongue	11.1% (n=1)	0.053
	Base of tongue	11.1% (n=1)	
	Tonsil	44.4% (n=4)	
	Posterior pharyngeal wall	33.3% (n=3)	
Age	50-59	22.25% (n=2)	0.09
	60-69	22.25% (n=2)	
	70-79	55.5% (n=5)	
Gender	Male	100% (n=9)	0.328
	Female	0% (n=0)	
Variants	SCC, NOS	55.5% (n=5)	0.000
	Basaloid SCC	44.5% (n=4)	
Grade	Grade 2	77.7% (n=7)	0.008
	Grade 3	22.2% (n=2)	
Alcoholism	Alcoholic	100% (n=9)	0.104
	Non alcoholic	0% (n=0)	
Smoking	Smoker	100% (n=9)	0.185
	Non smoker	0% (n=0)	
Betel nut chewing	Chewer	55.5% (n=5)	0.8
	Non chewer	44.5% (n=4)	
Stage of disease	Stage I	22.2% (n=2)	0.395
	Stage II	11.1% (n=1)	
	Stage III	11.1% (n=1)	
	Stage IV	55.5% (n=1)	

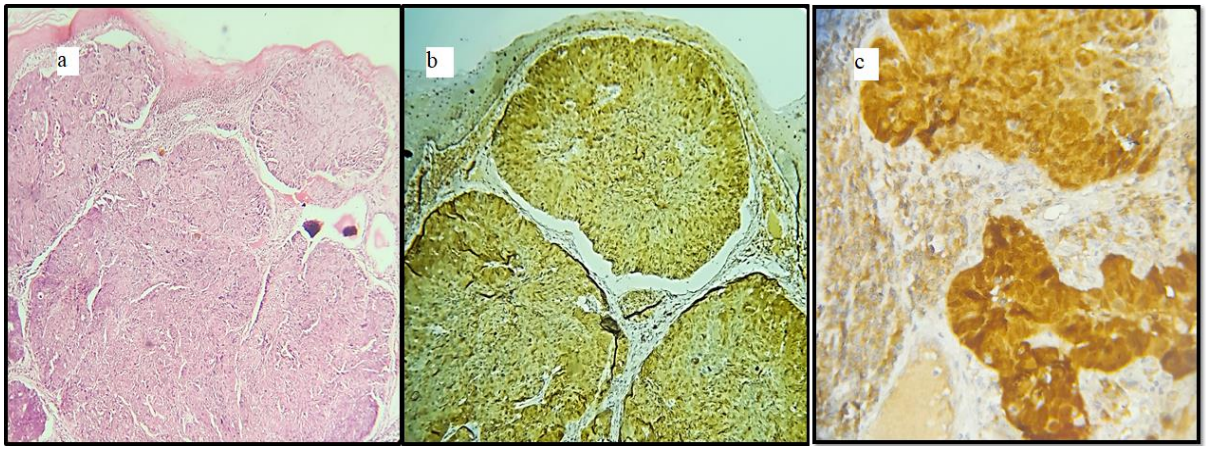

Figure 1: (A) Moderately differentiated squamous cell carcinoma (H and E, 10x) (B) P16 Immunohistochemistry, 10x (C) P16 strong (3+) nucleocytoplasmic staining, 40x.

P16 positive expression characterised by strong (2+/3+) nucleocytoplasmic or nuclear staining in >70% of cells, is observed only in 0.82% of OSCC and 9% of OPSCC(p=0.004). (Figure 1) The subsite of P16 positive OSCC was oral tongue and P16 positive OPSCC was tonsil and posterior pharyngeal wall. Mean age of P16 positive cases is 65.5 years and all P16 positive cases are males.

P16 positive expression showed significant association with Grade of tumor (p= 0.008). 77.7% of P16 positive tumours are Grade 2 tumours and 22.3% of P16 positive cases are Grade 3 tumours. P16 positive expression showed significant association with histological variants of SCC (p=0.00). 66.66% of Basaloid SCC was P16 positive. (Figure 2) There is no significant association between p16 expression and other variables. (Table 2)

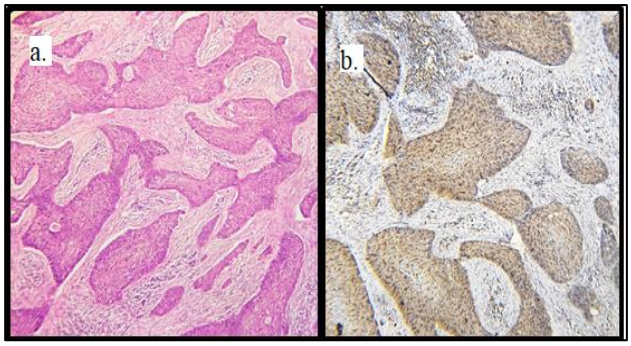

Figure 2: (A) Oropharynx- basaloid squamous cell carcinoma (H and E, 10x) (B) P16 IHC showing dense (2+) nuclear positivity (10x).

DISCUSSION

The overall positive P16 expression is observed only in 0.82% of OSCC and 9% of OPSCC in the present study. P16 was considered positive only when the malignant cells show moderate to strong, diffuse nuclear and cytoplasmic staining in at least 70% or more of the tumour. Cytoplasmic only staining, diffuse blush / weak intensity staining, and other focal and patchy patterns were considered negative. This finding is comparable with study conducted by Bhosale et.al, in central India who reported P16 positive expression in 1.4% of OSCC and 7.4% of OPSCC.[19] P16 positivity is much less when compared to similar studies in other states of India and central Asia. This might be due to reduced risk factors, as homosexuality and multiple sex partners are still considered a taboo in this society. There is significant association between P16 positive expression and site of the tumour (p=0.004).

The P16 overexpression is the surrogate marker of transcriptionally active HPV infection. p16INK4a is a cyclin-dependent kinase (CDK) inhibitor, encoded by the CDKN2A locus. pRb inactivation by HPV E7 oncogene causes upregulation of CDKN2A and thereby p16 overexpression. Detection of elevated p16 protein levels by immunohistochemistry (IHC) is the most widely used technique for HPV detection in the clinical setting. In HPV-unrelated HNSCC, mutation of the pRb-pathway is uncommon and p16 expression is low.[20]

Most of HPV-related head and neck squamous cell carcinoma arises in the oropharynx, particularly palatine and lingual tonsils due to the specialised reticulated lymphoepithelium through which HPV gains access.[21] In the present study, the subsite of P16 positive OSCC is the oral tongue, while the highest incidence of p16 positive tumors was seen in the tonsil (44.5%). There is no significant association between P16 positive expression and subsite with oral cavity and oropharynx (p=0.053), which was in concordance with the study of Ralli et al.[22]

In the present study, mean age of P16 positive SCC was 65.5 years, with highest incidence among the age group of 70-79 years. This is in contrast to documented younger age group of P16 positive SCC, with median age of diagnosis at 50-56 years. This might be the reflection of the higher age group of study participants. No significant association between age and P16 positive expression was observed in OSCC and OPSCC in this study.

All P16 positive cases were males, with no significant association observed between sex and P16 positive expression. Similar male predilection with no significant statistical association is also observed in study by Murthy et. al and Ralli et al.[3,22] Both HPV positive and negative squamous cell carcinoma have a male predilection.

In OPSCC, there are 6 cases of Basaloid SCC, out of which 4 casas (66.6 %) were P16 positive. There is significant association between basaloid variants of SCC and P16 positive expression. (p=0.00) Standard nomenclature is unsatisfactory for describing HPV mediated oropharyngeal cancers. The recommended terminology is “oropharyngeal squamous cell carcinoma, non-keratinizing type”. Here, “nonkeratinizing” is a low-power descriptor. Individual cell keratinization and tumor maturation seen on higher power, is compatible with the “nonkeratinizing” descriptor. The terms “poorly differentiated” and “basaloid squamous cell carcinoma” should not be used for this entity. The other morphological variants of HPV related OPSCC includes papillary, adenosquamous, lympho- epithelial, sarcomatoid, and small-cell carcinoma. Tumours resembling basaloid SCC and tumours with populations of ciliated cells have also been described.[2,21]

In this study, 110/210 (52.4%) were Grade 1 tumours, none of which showed positive P16 expression. Of the 9 P16 positive tumours, 77.7% (n=7) are grade 2 tumours and 22.3% (n=2) are grade 3 tumours with significant association between Grade of tumor and P16 positive expression (p= 0.008). this finding is comparable with study by Ralli et al, which also showed a significant correlation with histological grade of the tumor (P = 0.045). Grade is a strong and independent factor which helps to identify patients at high risk for distant metastasis, warranting efficient chemotherapy.[22] Histopathologic grading according to World Health Organization criteria, based on flattened polyhedral, round, or ovoid epithelial cells, intracellular or extracellular keratinization; and intercellular bridge is only applicable for P16 negative HPV unrelated SCC. Grading is of no relevance in HPV related Squamous cell carcinoma. HPV-positive OPSCC carries a favourable prognosis with a 5-year survival rates of 75–80%, when compared to survival rates of less than 50% among patients with similarly staged HPV negative tumors.[23]

The risk profile for HPV-positive oropharyngeal carcinomas differs from that for HPV-negative tumors. Sexual behaviour is an established risk factor for HPV positive tumours, with lifetime number of oral sex partners being the most strongly associated factor.

Other risk factors include open mouth kissing, increased number of vaginal or any sex partners, aged less than 18 years at the time of first oral sex, and history of cervical HPV infection.[24] One of the limitations of this study is that the sexual behaviour of the study population could not be analysed owing to ethical concerns.

All the study participants with P16 positive OSCC and OPSCC were alcoholics and smokers. HPV-positive tumors are less likely to arise in individuals with heavy tobacco and alcohol exposure. More research is needed to validate on the interactions between HPV, tobacco, and alcohol as tobacco smoking is associated with higher oral HPV prevalence in many studies.[25]

However, there was no significant association between p16 positive expression and any of the risk factors. In the study by Ralli et al22, statistically significant association between p16 expression and patients with history of paan chewing was observed (p=0.03).

Majority of P16 positive SCC presented at Stage IV of disease, with high nodal status. Similar high N stage was observed in studies by Murthy et al and Ralli et al.[3,22] HPV positive OPSCC have a high propensity for nodal metastasis due to the anatomic structure of the tonsillar crypts with ample access to lymphatics. Hence, they often present with early T stage (T1–T2) and advanced N stage (N2b–N3) with an aggressive node metastasis and higher stage of disease.[26]

However, no significant association was found between P16 positivity and stage of tumour.

CONCLUSION

The present study was done to find out P16 positive OSCC and OPSCC in our setting as the published literature regarding prevalence of HPV in Kerala is limited. A total of 122 cases of OSCC and 88 cases of OPSCC were studied. The frequency of P16 positive expression in OSCC is 0.82% and in OPSCC is 9% in this study. P16 positive expression showed significant association with the site of tumor (p=0.004), Grade of tumor (p=0.008) and histological variant, basaloid SCC. (p=0.00). There was no significant association between P16 expression and other variables. P16 positivity is much lesser when compared to studies from other parts of India. However, further evaluation with HPV-PCR and ISH are needed to confirm the HPV infection.

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