Original Article

Evaluation of p16 expression in oral and oropharyngeal squamous cell carcinoma

Pallavi Saxena, Sruthi Prasad

Department of Pathology, Kempegowda Institute of Medical Sciences, Bengaluru, Karnataka, India

Abstract

Introduction: Oral and oropharyngeal cancers together are the 6th most common cancers in the world and more than 90% are squamous cell carcinomas (OSCC, OPSCC). HPV is an important risk factor. p16 expression apart from indirectly assessing HPV infection, is an independent favorable prognostic marker. Lewis/Modified Lewis criteria of p16 grading identifies a subset of patients with improved overall survival.

Aims: (1) To evaluate p16 expression in these cancers. (2) To correlate p16 expression with age, gender, sub-site, histological type and grade. (3) To utilize the Lewis/modified Lewis criteria.

Methodology: The study included 70 cases of OSSC's and OPSCC's. Histological features were analyzed. p16 expression was determined and graded. Results were analyzed and evaluated using Chi-square test (value of $P < 0.05$ was taken significant).

Results: p16 positivity was seen in 46/70 (66%) cases (44 OSCC & 26 OPSCC). It was more frequent in younger patients and significantly higher in males. There was no correlation between degree of differentiation and p16 expression. In OSCC, 72.7% were p16 positive, mostly from tongue, buccal mucosa, and hard palate. p16 positivity was seen in 53.8% of OPSCCs, mostly from base of tongue and tonsil. Also, 30.4% of all cases could be included in Lewis criteria and 39.1% in modified Lewis criteria.

Conclusion: p16 is an inexpensive, easily available marker, it may be incorporated routinely in all histologically diagnosed cases of OSCC and OPSCC.

Keywords: HPV, Lewis/modified Lewis criteria, OPSCC, OSCC, p16

Address for correspondence: Dr. Sruthi Prasad, Associate Professor, Department of Pathology, Kempegowda Institute of Medical sciences, Bengaluru, Karnataka, India.
E-mail: Sruthi.chandra@gmail.com
Submitted: 23-Jun-2022, **Accepted:** 19-Jul-2022, **Published:** 17-Oct-2022

INTRODUCTION

Oral and oropharyngeal cancers, are sixth most common cancers in the world, with >90% being squamous cell carcinomas (OSCC, OPSCC).[1-4] HPV (Human Papilloma virus) is a major risk factor and can be assessed through p16 expression.[5,6] HPV-positive cancers have a better prognosis, even with more advanced disease.[7] p16 is also an independent favorable prognostic factor irrespective of HPV status.[8]

Access this article online

Quick Response Code:

Website: www.jomfp.in

DOI: 10.4103/jomfp.jomfp_278_22

The study evaluates p16 expression in these cancers and correlates it with age, gender, site/type of tumor and degree of differentiation. We have incorporated Lewis/modified Lewis criteria, to stratify them into prognostically favorable groups.[9]

MATERIALS AND METHODS

This was an observational study, from July 2017 to June 2019 on a sample size of 70. It included all biopsies and

How to cite this article: Saxena P, Prasad S. Evaluation of p16 expression in oral and oropharyngeal squamous cell carcinoma. J Oral Maxillofac Pathol 2022;26:376-81.

resected specimens received in the department of pathology from patients with clinically suspected oral (tongue, buccal mucosa, gingivobuccal sulcus, retromolar trigone, hard palate, and floor of mouth) and oropharyngeal (base of tongue, tonsil, vallecula, posterior pharyngeal wall, and soft palate) cancer. Relevant clinical details were taken. Ethical clearance has been obtained by the Institutional ethics committee.

All specimens/biopsies were fixed in 10% neutral buffered formalin (NBF) and subjected to routine processing for paraffin embedding. Two sections were cut from each block, one (4-5 μ) stained with Hematoxylin and Eosin (H&E) while the other (3 μ) on poly-L-lysine slide for p16 immunohistochemical analysis. The study included all cases of squamous cell carcinomas (SCC), dysplasia, and carcinoma in situ. The SCC's were categorized as per the W.H.O classification and the conventional SCCs were graded from well to poorly differentiated. Exclusion criteria were inadequate biopsy and benign lesions.

Immunohistochemical staining for p16INK4a was performed with both positive and negative controls, using Biogenex Lifesystems Histology Kit (G175-405 Clone), in accordance with the manufacturer's instructions.

Interpretation of p16 staining: Sections showing both complete (nuclear and cytoplasmic) and/or partial (only cytoplasmic) staining in >= 5% cells were considered positive. Sections showing no staining or staining in <5% cells were considered as negative. The p16-positive slides were graded semi-quantitatively in a quartile manner into four grades based on percentage of cells showing positive immunostaining as Grade1 (up to 25%), Grade 2 (26--50%), Grade 3 (51--75%), and Grade 4 (>75%). They were further assessed for degree of confluence (defined as group of 10 contiguous cells with positive immunostaining) in a semi-quantitative quartile manner (up to 25% grade 1, 26--50% grade 2, 51--75% grade 3 and >75% grade 4). We further applied the Lewis criteria (>75% p16-positive cells OR > 50% p16-positive cells with >25% confluence) and modified Lewis criteria (>75% p16-positive cells OR 26--75% positive cells with >75% confluence) to identify patients, who might have an increased overall survival and better prognosis.

The data was entered in Microsoft Excel master sheet and analyzed using Statistical Package for Social Sciences (SPSS) version 17.0 software. A value of $P < 0.05$ was taken as significant.

RESULTS

A total 75 cases were evaluated, of which 70 cases were included in the study. Of the five cases excluded, two were benign lesions and three were inadequate biopsies. The 70 cases included histologically diagnosed cases of OSCC (44/70, 63%) and OPSCC (26/70, 37%).

The age of the patients in the study ranged from 31 to 95 years with a mean of 56.9 ± 15.6 years, and a peak between 40 to 60 years (47.1%). Among OSCC, the age ranged from 31 to 95 years with a mean of 53.8 ± 16.1 years and a peak between 40 to 60 years age (52.3%). Whereas in OPSCC, the age ranged from 35 to 85 years with a mean of 62.0 ± 13.5 years and a peak above 60 years (57.7%).

Most of the patients were males 47/70 (67%), with females accounting for 23/70 (33%) cases and showing a male: female ratio of 2:1. Patients with OSCC comprised of 27 males (61.4%) and 17 females (38.6%) with a male: female ratio of 1.6:1 and those with OPSCC comprised of 20 males (76.9%) and 6 females (23.1%) with a male: female ratio of 3.3: 1.

The most common sub-site in OSCC was tongue 16/44 (36.4%), followed by buccal mucosa 13/44 (29.5%) and GBS 9/44 (20.5%). The other sites were hard palate 3/44 (6.8%), floor of mouth 2/44 (4.5%) and retromolar trigone 1/44 (2.3%). Among the OPSCCs, the most common sub-site involved was base of the tongue 10/26 (38.5%), followed by tonsil 9/26 (34.6%) and vallecula 4/26 (15.4%). The other sites were soft palate 2/26 (7.7%) and posterior pharyngeal wall 1/26 (3.8%).

Histological grading was done for all cases. Of the 70 cases, 41 (58.6%) were MDSCC, 18 (35.7%) WDSCC, and 5 (7.1%) PDSCC [Figure 1]. There were two (2.9%) cases each of Adenosquamous carcinoma, Basaloid SCC, and Dysplasia.

In OSCCs, 25/44 (56.8%) were MDSCC, 13/44 (29.5%) WDSCC, 4/44 (9.1%) PDSCC, and 2/44 (4.6%) were Adenosquamous carcinoma. In OPSCCs, 16/26 (61.5%) were MDSCC, 5/26 (19.2%) WDSCC, 1/26 (3.8%) was PDSCC and two cases each (7.7%) were Basaloid SCC and dysplasia.

The p16 IHC showed that 24/70 (34%) cases were negative, while 46/70 (66%) were positive. Of the 44 cases of OSCC, 32 (72.7%) were p16 positive, while 12 (27.3%) were negative. Of the 26 cases of OPSCC, 14 (53.8%) were p16 positive, while 12 (46.2%) were negative.

Overall (both OSCC and OPSCC) p16 positivity was seen in a younger subset of patients (mean age 54.4 years), than p16 negative ones (mean age 61.9 years). Though, p16-positive cases had a lower mean age (52 yrs and 60.9 yrs) as compared to p16-negative cases (58.7 yrs and 63.3 yrs), it was not statistically significant. Overall, p16 positivity was seen in 39.1% (9/23) female and 78.7% (37/47) male patients. Thus, a statistical significance ($P = 0.001$) was seen between male gender and p16 positivity.

In OSCC, p16 positivity was seen in 35.3% (6/17) female and 96.3% (26/27) male patients. Thus, a statistical significance ($P < 0.001$) could be seen between the male gender and p16 positivity. Whereas in OPSCC, p16 positivity was seen in 50% (3/6) female patients and 55% (11/20) male patients. However, this was not statistically significant.

When histological differentiation and p16 was considered among OSCC, p16 positivity was seen in 22/25 (88%) MDSCC, 2/4 (50%) PDSCC, 7/13 (53.9%) WDSCC, and 1/2 (50%) of adenosquamous carcinoma. Among OPSCC, p16 positivity was seen in 8/16 (50%) MDSCC, 1/1 (100%) PDSCC, 4/5 (80%) WDSCC, and 1/2 (50%) Basaloid SCC. There was no statistical correlation between degree of differentiation and p16.

When sub-site evaluation of p16 positivity was considered, among OSCC it was seen that 13/16 (81.3%) from the tongue were p16 positive, followed by 8/13 (61.5%) in buccal mucosa and 5/9 (55.6%) in GBS. All cases from hard palate 3/3 (100%), retromolar trigone 1/1 (100%), and floor of mouth 2/2 (100%) were p16 positive. In OPSCC, 7/10 (70%) cases from base of tongue were p16 positive, followed by 4/9 (44.4%) in tonsil, 1/2 (50%) in soft palate, and 1/4 (25%) in vallecula. The single case from PPW 1/1 (100%) was p16 positive.

The quartile method of IHC grading was applied to the p16-positive cases and when confluence grading was applied to the same it was seen that 9/32 (28%) OSCC and 5/14 (35.7%) OPSCC fit in Lewis criteria [Figures 2 and 3]. Also, 11/32 (34%) OSCC and 7/14 (50%) OPSCC fit in modified Lewis criteria. [Table 1].

DISCUSSION

Oral and Oropharyngeal cancers together are the sixth most common cancers in the world, accounting for 4% of all malignancies in males and 2% in females.[1-3] Oral cancer is among the top three cancers in India, while OPSCC represents 10--15% of all head and neck cancers.[5,10] The risk factors associated are alcoholism, smoking, tobacco, and HPV.[11] In India, more than 90% of oral cancer cases

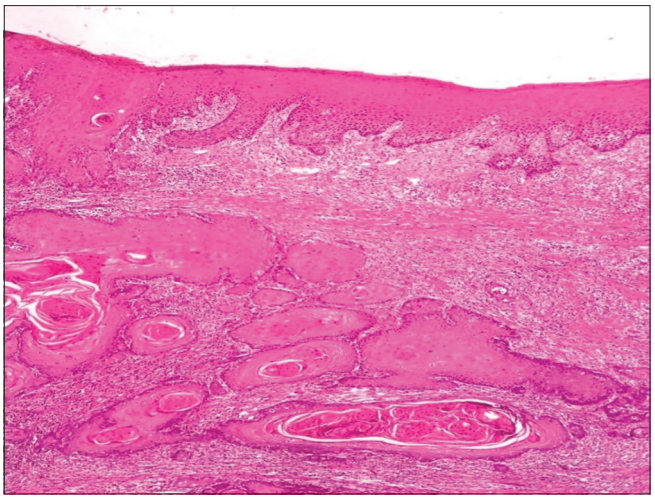

Figure 1: WDSCC - Nests of neoplastic squamous cells with many keratin pearls (H&E,40x)

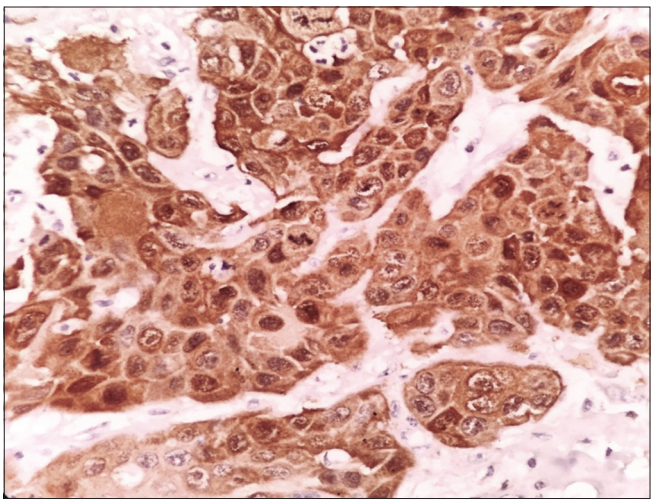

Figure 2: p16 IHC, showing both partial and complete staining and confluent staining (400x)

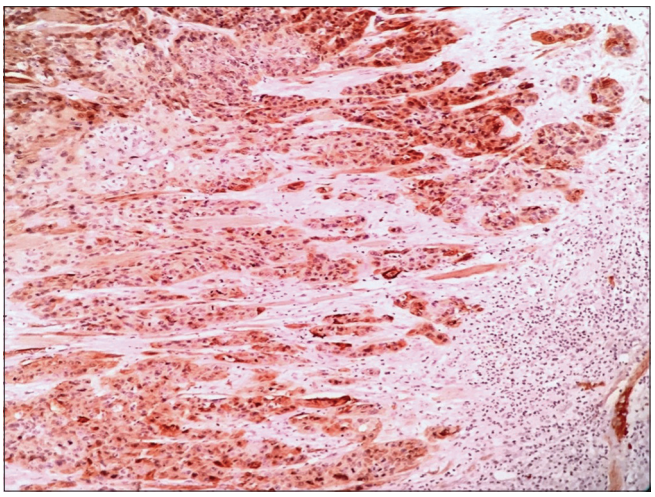

Figure 3: p16 IHC, Grade III showing positivity in 51-75% tumor cells with >75% confluence (40x)

Table 1: p16 Grading for Lewis and Modified Lewis criteria

p 16 Grading	Confluence, <25%	Confluence, 26-50%	Confluence 51-75%	Confluence, >75%
Grade 1 (upto25% positivity)				
OSCC, *n*=12	10/12 (83.3%)	2/12 (16.7%)	0	0
OPSCC, *n*=5	5/5 (100%)	0	0	0
Grade 2 (26-50% positivity)				
OSCC, *n*=11	2/11 (18.2%)	4/11 (36.4%)	4/11 (36.4%)	1/11 (9%)
OPSCC, *n*=4	1/4 (25%)	0	1/4 (25%)	2/4 (50%)
Grade 3 (51-75% positivity)				
OSCC, *n*=7	0	0	3/7 (42.9%)	4/7 (57.1%)
OPSCC, *n*=3	0	0	1/3 (33.3%)	2/3 (66.7%)
Grade 4 (>75%)				
OSCC, *n*=2	0	0	0	2/2 (100%)
OPSCC, *n*=2	0	0	0	2/2 (100%)

are attributed to use of tobacco products, additionally, tobacco use appears to be strongly associated with oral HPV infection.[11-14] HPV infection is more prominent in OSCC cases from India, than from other countries.[4,5] HPV-associated OPSCC has increased more than 2 fold over the last two decades.[15,16] HPV-positive OPSCC and OSCC patients have better prognosis and are characterized by p16 overexpression.[7] p16 evaluation is not only an indirect method of HPV detection, but also an independent favorable prognostic factor (irrespective of HPV status) associated with better response to treatment and overall survival.[9,17]

The present study included 70 SCCs (44 OSCC and 26 OPSCC). Histopathology showed mostly WDSCC (18/70, 25.7%) and MDSCCs (41/70, 58.6%). p16 positivity was seen in a total of 46/70 (66%) cases. Whereas, studies by Ralli *et al.*[10] on head and neck cancers showed p16 positivity in 59/75 (78.7%). Lassen *et al.*[17] and Murthy *et al.*[18] in their studies on head and neck cancers noted that p16 positivity was seen in younger patients (57 and 53 years, respectively), when compared to p16 negative ones (60 and 57 years, respectively). This is similar to our study where overall p16 positivity was seen in younger patients (mean age—54.4 years p16 positive and mean age—61.9 years p16 negative). The current study showed a statistically significant correlation between male gender and p16 positivity (78.7% male and 39.1% female), similar to studies by Ralli *et al.*[10] (79.7% males and 72.7% females) and Smith *et al.*[19] (42.1% males and 30.4% females). When histological differentiation was taken into account, Lassen *et al.*[17] noted lower p16 positivity 16/91 (17.6%) in WDSCC and MDSCC. Whereas, in the current study p16 positivity was seen in 11/18 (61.1%) of WDSSC and 32/41 (78%) MDSCC. Both the studies (including the present study) did not demonstrate any statistical significance.

Oral SCC: The common subsites of OSCC were tongue 16/44, 36.4%; buccal mucosa 13/44, 29.5% and GBS 9/44, 20.5%; which is similar to findings by Yang H *et al.*[20] (tongue 99/145, 68.3%; GBS 23/145, 15.9%; buccal mucosa 10/145, 6.9%).

p16 positivity was seen in 32/44 (72.7%) OSCCs. Pathak *et al.*[8] and Dragomir *et al.*[21] showed p16 positivity in majority of cases (31/50, 62% and 22/34, 64.7% respectively). Present study noted p16 positivity in younger patients (52 years) like Salas *et al.*[22] (48.67 years); as compared to p16 negative ones (current study 58.7 years, Salas *et al.* 63.71 years). A statistically significant association between male gender and p16 positivity has also been noted. Sritippho *et al.*,[23] also showed a higher p16 positivity in males (6/19, 31.6%) when compared to females (4/22, 18.2%). However, Babiker *et al.*[3] noted that p16 positivity was higher in females (13/24, 54.2%) than males (27/76, 35.5%). Both the studies were statistically insignificant.

When histological differentiation was considered, there was no association between p16 and degree of differentiation in the current study. Babiker *et al.*[3] noted statistically significant p16 expression in 12/22 (54.5%) cases of WDSCC, with lower association in MDSCC (16/34, 47.1%) and PDSCC (12/44, 27.3%). However, Sritippho *et al.*[23] noted p16 positivity in 6/16 (37.5%) cases of WDSCC, while MDSCC and PDSCC cases did not show p16 positivity. Also, 1/2 (50%) cases of Adenosquamous carcinoma showed p16 positivity in the present study, similar to findings of Masand *et al.*[24] (2/4, 50% p16 positive). When subsite of OSCC and p16 expression are considered, the current study showed no statistical significance. However, the study done by Zafereo *et al.*[25] showed a statistical correlation with p16 expression being predominant in cases from the tongue [Table 2]. The other studies (Sritippho *et al.*, Yang *et al.*)[4,20] showed no statistical significance. However, on review of results in all the studies, it is noted that a major proportion of cases from tongue, hard palate and buccal mucosa show p16 expression. [Table 2].

Oropharyngeal SCC: OPSCCs, were mostly seen in the base of tongue 10/26 (38.5%) and tonsil 9/26 (34.6%),

similar to findings concluded by Broglie *et al.*[7] (tonsil 68/118, 57.6%; base of tongue (50/118, 42.4%).

p16 positivity was seen in 14/26 (53.8%) cases of OPSCC, which is comparable to findings by Shinohara *et al.*[26] (29/53, 54.7%) and lower than those of Samuel *et al.*[9] (48/81, 59.3%). p16-positive patients were younger (60.9 yrs) when compared to p16-negative patients (63.3 yrs). This is similar to findings of Shinohara *et al.*[26] and Barasch *et al.*[9] where the mean age was lower in p16-positive patients (61 yrs and 53.5 yrs, respectively) in contrast to p16-negative ones (67.5 yrs and 62 yrs, respectively). However, unlike the present study, both these studies showed statistical significance.[9,26] Other findings of Fischer CA *et al.*[27] and Broglie (p16 positive 60.4 yrs and 63 yrs, p16 negative 58 yrs and 60 yrs, respectively) *et al.*[7] were not in concordance with the present study, since they showed a higher mean age in p16-positive patients. Gender wise, p16 positivity was seen in a higher number of males 3/6 (50%), when compared to females 11/20 (55%). This is similar to findings of Barasch *et al.*[9] with higher p16 positivity in males (35/57, 61.4%) compared to females (13/24, 54.2%). Whereas, Shinohara S *et al.*,[26] noted that p16 positivity was higher in female (8/11, 72.7%)) than male (21/42, 50%) patients. Both the studies had shown no statistical significance.[9,26]

When histological differentiation was considered, the present study had a higher number of WDSCC (4/5, 80%) and MDSCC (8/16, 50%) showing p16 positivity. These observations were unlike those seen by Weinberger *et al.*[28] where, p16 positivity was seen in zero cases of WDSCC, 5/53 (9.4%) MDSCC, and 14/45 (31.1%) PDSCC. They also found this to be statistically significant, unlike the present study. While, 1/2 (50%) cases of Basaloid SCC showed p16 positivity, Chernock *et al.*[29] showed 8/12 (66.7%) cases of Basaloid SCC to be p16 positive. When subsite and p16 expression was taken into account, all three studies (Barasch *et al.*,[9] Broglie *et al.*,[7] current study) could not find any statistical significance [Table 3]. However, what has been similar in all studies is that a good proportion of cases from the base of tongue and tonsil showed p16 expression. [Table 3].

It was seen that 14/46 (30.4%) cases could be included in Lewis criteria (OSCC—9/32, 28%; OPSCC—5/14, 35.7%), while 18/46 (39.1%) cases could be included in modified Lewis criteria (OSCC—11/32, 34%, OPSCC—7/14, 50%). Though the present study hasn't evaluated the follow up of patients, it is known that these patients have an overall improved survival and better response to treatment.[9] Applying these criteria is simple, but additional research with larger numbers, evaluating follow up, response to therapy and survival is needed to determine the prognosis of these subsets of patients.

Table 2: OSCC - Comparison of p16 expression and various sub-sites reported by various authors with our study

Study	P16 Positive (*n*,%)	P16 Negative (*n*,%)	*P*
Sritippho T *et al*. (*n*=41) 2016			
Tongue (*n*=12)	3 (25%)	9 (75%)	>0.05
Buccal mucosa (*n*=6)	2 (33.3%)	4 (66.7%)	
Gingival/Alveolar mucosa (*n*=17)	3 (17.6%)	14 (82.4%)	
Hard palate (*n*=3)	2 (66.7%)	1 (33.3%)	
Others (*n*=3)	0	3 (100%)	
Zafereo ME *et al*. (*n*=210) 2016			
Tongue (*n*=129)	47 (36.4%)	82 (63.6%)	0.021
GBS (*n*=55)	10 (18.2%)	45 (81.8%)	
Floor of mouth (*n*=26)	5 (19.2%)	21 (80.8%)	
Yang H *et al*. (*n*=145) 2018			
Tongue (*n*=99)	28 (28.3%)	71 (71.7%)	>0.05
Buccal mucosa (*n*=10)	2 (20%)	8 (80%)	
Alveolar ridge (*n*=23)	5 (21.7%)	18 (78.3%)	
Floor of mouth (*n*=13)	1 (7.7%)	12 (92.3%)	
Present study (*n*=44)			
Tongue (*n*=16)	13 (81.3%)	3 (18.7%)	
Buccal mucosa (*n*=13)	8 (61.5%)	5 (38.5%)	0.541
GBS (*n*=9)	5 (55.6%)	4 (44.4%)	
Hard palate (*n*=3)	3 (100%)	0	
Floor of mouth (*n*=2)	2 (100%)	0	
Retromolar trigone (*n*=1)	1 (100%)	0	

Table 3: OPSCC - Comparison of p16 expression and various sub-sites reported by various authors with our study

Study	P16 Positive (*n*, %)	P16 Negative (*n*, %)	*P*
Brogie MA *et al*. (*n*=124) 2013			
Base of tongue (*n*=50)	22 (44%)	28 (56%)	0.46
Tonsil (*n*=68)	31 (45.6%)	37 (54.4%)	
PPW/soft palate (*n*=6)	1 (16.7%)	5 (83.3%)	
Barasch S *et al*. (*n*=81) 2016			
Base of tongue (*n*=30)	20 (66.7%)	10 (33.3%)	0.8
Tonsil (*n*=42)	27 (64.3%)	15 (35.7%)	
PPW/soft palate (*n*=9)	1 (11.1%)	8 (88.9%)	
Present study (*n*=26)			
Base of tongue (*n*=10)	7 (70%)	3 (30%)	0.466
Tonsil (*n*=9)	4 (44.4%)	5 (55.6%)	
PPW (*n*=1)	1 (100%)	0	
Soft palate (*n*=2)	1 (50%)	1 (50%)	
Vallecula (*n*=4)	1 (25%)	3 (75%)	

To conclude, OSCC and OPSCC are common in the age of 40--60 yrs with a male predominance. p16 positivity was seen in 72.7% cases of OSCC and 53.8% cases of OPSCC. It is seen in a younger age group, more in males and has no association with degree of differentiation. p16 expression was more often noted in tongue, buccal mucosa, hard palate in OSCCs, and among OPSCCs in base of tongue and tonsil. p16 is a simple and inexpensive prognostic marker which can be routinely evaluated in these cancers.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Sloan P, Gale N, Hunter K, Lingen M, Nylander K, Reibel J, *et al*. Squamous cell carcinoma. In: El-Naggar AK, Chan JKC, Grandis JR, Takata T, Slootweg PJ, editors. WHO Classification of Head and Neck Tumours. 4th ed, vol 9. Lyon, France: IARC Press; 2017. p. 109–11.
2. Warnakulasuriya S. Global epidemiology of oral and oropharyngeal cancer. Oral Oncol 2009;45:309-16.
3. Babiker AY, Rahmani AH, Abdalaziz MS, Albutti A, Aly SM, Ahmed HG. Expressional analysis of p16 and cytokeratin19 protein in the genesis of oral squamous cell carcinoma patients. Int J Clin Exp Med 2014;7:1524–30.
4. Sritippho T, Chotjumlong P, Iamaroon A. Roles of human papillomaviruses and p16 in oral cancer. Asian Pac J Cancer Prev 2015;16:6193-200.
5. Patil S, Rao RS, Amrutha N, Sanketh DS. Analysis of human papilloma virus in oral squamous cell carcinoma using p16: An immunohistochemical study. J Int Soc Prev Community Dent 2014;4:61-6.
6. Westra WH, Lewis JS Jr. Update from the 4th Edition of the World Health Organization Classification of Head and Neck Tumours: Oropharynx. Head and Neck Pathol 2017;11:41–7.
7. Broglie MA, Soltermann A, Rohrbach D, Haile SR, Pawlita M, Studer G, *et al*. Impact of p16, p53, smoking, and alcohol on survival in patients with oropharyngeal squamous cell carcinoma treated with primary intensity-modulated chemoradiation. Head Neck 2013;35:1698-706.
8. Pathak A, Singh M, Agarwal A, Amit S. Determination of p16 overexpression as an indicator of human papillomavirus infection in oral dysplasia and carcinoma. Indian J Dent Res 2017;28:418-23.
9. Barasch S, Mohindra P, Hennrick K, Hartig GK, Harari PM, Yang DT. Assessing p16 status of oropharyngeal squamous cell carcinoma by combined assessment of the number of cells stained and the confluence of p16 staining: A validation by clinical outcomes. Am J Surg Pathol 2016;40:1261-9.
10. Ralli M, Singh S, Yadav SPS, Sharma N, Verma R, Sen R. Assessment and clinicopathological correlation of p16 expression in head and neck squamous cell carcinoma. J Can Res Ther 2016;12:232-7.
11. Varshitha A. Prevalence of oral cancer in India. J Pharm Sci Res 2015;7:845-8.
12. Morbini P, Benazzo M. Human papillomavirus and head and neck carcinomas: Focus on evidence in the label of published data. Acta Otorhinolaryngol Ital 2016;36:249-58.
13. Fakhry C, Gillison ML, D'Souza G. Tobacco use and oral HPV-16 infection. JAMA 2014;312:1465-7.
14. Ducatman BS. The role of human papillomavirus in oropharyngeal squamous cell carcinoma. Arch Pathol Lab Med 2018;142:715–8.
15. Lingen MW. Head and neck. In: Kumar V, Abbas AK, Aster JC, editors. Robbins and Cotran Pathologic Basis of Disease. 9th ed, vol 2. Elsevier Inc; 2015. p. 727-48.
16. D'Souza G, Kreimer AR, Viscidi R, Pawlita M, Fakhry C, Koch WM, *et al*. Case-control study of human papillomavirus and oropharyngeal cancer. N Engl J Med 2007;356:1944-56.
17. Lassen P, Eriksen JG, Hamilton-Dutoit S, Tramm T, Alsner J, Overgaard J. Effect of HPV-associated p16INK4A expression on response to radiotherapy and survival in squamous cell carcinoma of the head and neck. J Clin Oncol 2009;27:1992-8.
18. Murthy V, Swain M, Teni T, Pawar S, Kalkar P, Patil A, *et al*. Human papillomavirus/p16 positive head and neck cancer in India: Prevalence, clinical impact, and influence of tobacco use. Indian J Cancer 2016;53:387-93.
19. Smith EM, Rubenstein LM, Hoffman H, Haugen TH, Turek LP. Human papillomavirus, p16 and p53 expression associated with survival of head and neck cancer. Infect Agent Cancer 2010;5:4-13.
20. Yang H, Cao Y, Li ZM, Li YJ, Jiang WQ, Shi YX. The role of protein $p16^{INK4a}$ in non-oropharyngeal head and neck squamous cell carcinoma in Southern China. Oncol Lett 2018;16:6147-55.
21. Dragomir LP, Simionescu C, Margaritescu C, Stepan A, Dragomir IM, Popescu MR. P53, p16 and Ki67 immunoexpression in oral squamous carcinomas. Rom J Morphol Embryol 2012;53:89-93.
22. Salas E, Sanchez P, Postigo J, Castaneda CA, Castillo M, Villegas V, *et al*. Nodal involvement and p16-staining in upper alveolar ridge and hard palate cancer. J Cancer Metastasis Treat 2018;4:1-15.
23. Sritippho T, Pongsiriwet S, Lertprasertsuke N, Buddhachat K, Sastraruji T, Iamaroon A. p16-a Possible surrogate marker for high-risk human papillomaviruses in oral cancer? Asian Pac J Cancer Prev 2016;17:4049-57.
24. Masand RP, EI-Mofty SK, Ma XJ, Luo Y, Flanagan JJ, Lewis JS Jr. Adenosquamous carcinoma of the head and neck: Relationship to human papillomavirus and review of the literature. Head Neck Pathol 2011;5:108-16.
25. Zafereo ME, Xu L, Dahlstrom KR, Viamonte CA, El-Naggar AK, Wei Q, *et al*. Squamous cell carcinoma of the oral cavity often overexpresses p16 but is rarely driven by human papillomavirus. Oral Oncol 2016;56:47-53.
26. Shinohara S, Kikuchi M, Tona R, Kanazawa Y, Kishimoto I, Harada H, *et al*. Prognostic Impact of p16 and p53 expression in oropharyngeal squamous cell carcinomas. Jpn J Clin Oncol 2014;44:232-40.
27. Fischer CA, Kampmann M, Zlobec I, Green E, Tornillo L, Lugli A, *et al*. p16 expression in oropharyngeal cancer: Its impact on staging and prognosis compared with the conventional clinical staging parameters. Ann Oncol 2010;21:1961-6.
28. Weinberger PM, Yu Z, Haffty BG, Kowalski D, Harigopal M, Sasaki C, *et al*. Prognostic significance of p16 protein levels in oropharyngeal squamous cell cancer. Clin Cancer Res 2004;10:5684-91.
29. Chernock RD, Lewis JS Jr, Zhang Q, El-Mofty SK. Human papillomavirus-positive basaloid squamous cell carcinomas of the upper aerodigestive tract: A distinct clinicopathologic and molecular subtype of basaloid squamous cell carcinoma. Hum Pathol 2010;41:1016-23.