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Original Research Article

A study on Expression of Human Papillomavirus in Oral Squamous Cell Carcinomas using p16

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Conflict of interest: Nil

Abstract:

Aim and Background: The expression of human papillomavirus in oral squamous cell Carcinomas using p16. Oral cancer ranks first among all cancer cases in males, and it ranks third among females. More than 90% of oral cancers are oral squamous cell carcinomas. Infection with human papillomavirus (HPV) poor oral hygiene and dietary deficiencies have been identified as minor risk factors for developing carcinoma in the oral cavity.

Materials and Methods: This is a Cross – sectional observational study and conducted on composite resected specimens and biopsy specimens of suspected oral squamous cell carcinoma lesions received at Sri Venkateswara Medical College, Tirupati, from November 2019 to 2021. All oral cancer patients P16INK4A genes were analysed by using immunohistochemical method. The pattern of p16INK4a was categorized as positive or negative. Results: On P16INK4A immunostaining, 12 cases (24%) showed positive staining, and 38 cases (76%) showed negative staining. The correlation between P16 staining and histopathological grading of OSCC was statistically insignificant.

Conclusion: In the present study, P16INK4A was used as a surrogate marker to evaluate HPV association in OSCC. The findings demonstrated HPV positivity in a small proportion of cases (24%). This relatively low prevalence may be attributed to the limited sample size and the restricted geographic area included in the study. Consequently, further studies with larger sample sizes and broader geographic representation are warranted to better elucidate the association between HPV infection and OSCC.

Keywords: oral squamous cell Carcinomas, Human papillomavirus, Immuno histochemical method, P16INK4A.

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Introduction

In India, Oral cancer constitutes 9.8% of total cancer cases. According to GLOBOCON 2018, OSCC ranks 16, whereas, in India, OSCC ranks 2 with 119, 992 new cases and 72,616 deaths. Oral squamous cell carcinomas (OSCC) comprise malignant lesions arising from the mucosal epithelium of the oral cavity. It is most frequent in the fifth and sixth decades of life1. Oral cancer can affect any area of the oral mucosa. However, among many Asian populations, OSCC most commonly affects the buccal mucosa due to tobacco chewing and betel quid chewing [1].

The main etiological factors for OSCCs are tobacco and alcohol. Tobacco is used in various forms like betel quid, tobacco with lime, bidi, hookah, etc., in India and Indian subcontinent countries. However, in recent studies increase in oral cancer is reported without significant tobacco and alcohol exposure [2].

The association between high-risk HPV and oral cancer development dates back to 1983, but the presence of viral DNA was confirmed by in situ hybridizations (ISH) two years later. HPV-16 and HPV-18 are associated with oral squamous cell carcinoma [3]. HPV prevalence differs according to the geographical area, ethnicity, and sexual behaviour of the study population. Globally, the prevalence of Human papillomavirus-associated OSCC varies from 0-30% [4].

A cellular protein, P16, regulates the cell cycle and is expressed at very low levels. At this level, P16 is almost undetectable by IHC. However, in OSCC caused by HPV, P16 is strongly expressed in tumor cells due to the transforming activity of the E7 oncogene and makes it detectable by IHC [5].

Materials and Methods

Our study was a cross sectional and observational of oral squamous cell Carcinomas carried out at the department of Pathology, Sri Venkateswara medical college, Tirupati for a duration of the period one year from November 2019 to October 2021. A total of 45 biopsies/resected specimens of suspected oral squamous cell carcinomas sent in 10% buffered formalin were included. All the patients were selected based on the inclusion and exclusion criteria. All resected as well as biopsy specimens irrespective of age and gender were included in the study. Retrieval of tissue blocks from the resected specimens and histopathological slides of the corresponding numbers were collected. New slides were made from the corresponding blocks. The blocks were sectioned into 4 micron thick sections and stained with Hematoxylin – Eosin.

Then slides were examined under the microscope, and the diagnosis was made according to WHO classification. Those cases which met the inclusion criteria were subjected to immunohistochemical analysis for P16INK4A. The kit used is P16 mouse monoclonal antibody immunohistochemical staining obtained from Biogenex (Monoclonal Mouse Anti-Human P16, Clone-G175-405). Staining was done as per standard procedure. Evaluation of the immune-histochemical staining was done under light microscopy.

The pattern of p16INK4a was categorized as positive or negative. Positive p16INK4a was defined as the presence of nuclear staining or diffuse cytoplasmic staining, or both. Negative staining was defined as an absence of any staining in the tumor cells. A semi-quantitative assessment of immune-histochemical staining was done using Intensity Score and Proportion Score. The staining intensity ('Intensity Score') was graded as No staining -'0', Weak – '1', Moderate – '2', Strong -'3'. The percentage of tumor cells ('Proportion Score') stained was scored as score 0 - none of the cells stained, score '1' - 1% to 10% cells, score '2' - 11% to 50% cells score '3' - 51% to 80% cells stained, score '4' - 81% to 100% cells stained. A score of 0 was considered negative, and 1 to 4 was considered positive.

Data Analysis: All the data`s were collected and analysed by using S.P.S.S. Version 21 statistic software package. The data collected was analyzed and depicted in graphs and charts. Association between categorical variables was evaluated by using the Pearson chi-square test. A p-value of less than 0.05 was considered statistically significant.

Results

Age wise distribution of Oral squamous cell carcinomas patients: Table. 1 showed the majority of Oral squamous cell carcinomas patients were more than 50 years (70%). However, 26% of patients were between 40-50 years, and 4% were less than 40 years old.

Table 1: Age wise distribution of Oral squamous cell carcinomas patients

Age in years	No. of cases	Percentage
<40	02	4%
40-50	13	26%
>50	35	70%
Total	50	100%

Anatomical site in Oral squamous cell carcinomas cases: Table.2 showed the most of the carcinomas were from buccal mucosa (48%).16% were from anterior 2/3 of the tongue, 12% were from the palate (hard and soft), 8% were from retromolar trigone, 6% were from Gingivo buccal sulcus, 6% were from maxillary alveolus, 2% were from mandibular alveolus and 2% from lips.

Table 2: Anatomical site in Oral squamous cell carcinomas cases

Anatomical site	No. of cases	Percentage
Lips	1(lowerlip)	2%
Buccal mucosa	24	48%
Gingivo buccal sulcus	3	6%
Maxillary alveolus	3	6%
Mandibular alveolus	1	2%
Retro molar trigone	4	8%
Anterior 2/3 of the tongue	8	16%
Palate (Hard and soft)	6	12%
Total	50%	100%

Distribution of population with given HPE diagnosis: Table.3. indicates that the result shows that majority of the patients had well-differentiated squamous cell carcinoma (72%). 22% of the patients had moderately differentiated squamous cell

carcinoma, and 6% had poorly differentiated squamous cell carcinoma.

Table 3: Histopathological examination of Oral squamous cell carcinomas

Histopathological examination diagnosis	No. of cases	Percentage
Well-differentiated squamous cell carcinoma	36	72%
Moderately differentiated Squamous cell carcinoma	11	22%
Poorly differentiated Squamous cell carcinoma	03	6%
Total	50	100%

P16INK4A staining interpretation in OSCC cases

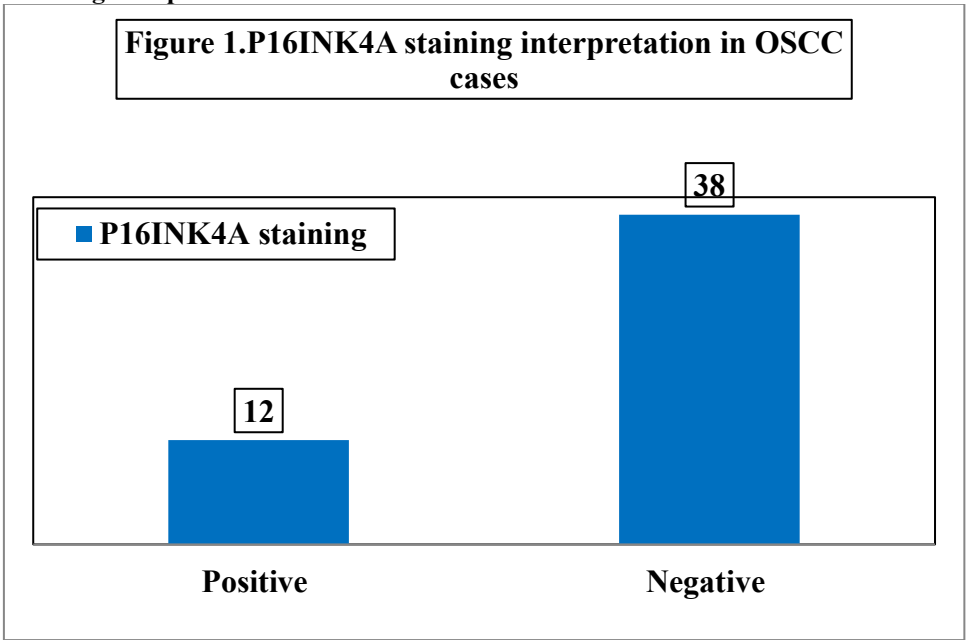


Figure .1 Observed that the 24% of the O.S.C.C. cases showed positive P16INK4A staining in our study.

Interpretation of percentage of cells showing P16INK4A positivity: Table.4 indicates that the our study, 9 (18%) cases showed 11-50% of cells stained for P16INK4A (positivity). 1 (2%) cases showed 51-80% of cells stained, 1 (2%) showed 1-10% of cells stained, and 1 (2%) case showed 100% of cells stained.

Table 4: Interpretation of percentage of cells showing P16INK4A positivity

Percentage of cells Showing P16 positivity	No. of cases	Percentage
1-10%	1	2
11-50%	9	18
51-80%	1	2
100%	1	2
Nil	38	76
Total	50	100%

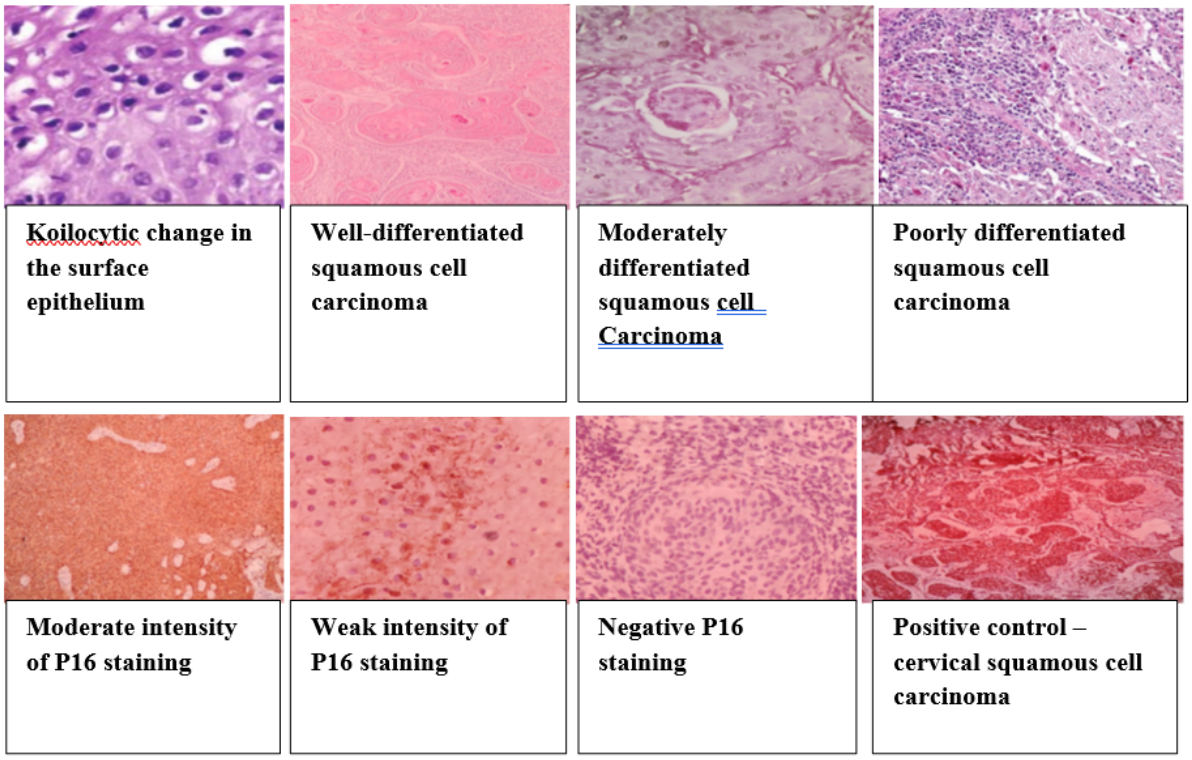
Grade calculated based on the percentage of cells stained for P16INK4A: Table.5 Regarding grade, based on the percentage of cells stained, 8 cases (16%) showed grade- 2 staining. 2 cases (4%) showed grade- 3, 1 case (2%) showed grade- 4 staining, and 1 case (2%) showed grade- 1 staining.

Grade of Staining	Number of Cases	Percentage
0	38	76
1	1	2
2	8	16
3	2	4
4	1	2
Total	50	100

Correlation between HPD and percentage of cells stained: Table 7 observed that the p-value of the test is 0.019 (<0.05), suggesting a significant correlation between histopathological grades of oral squamous cell carcinomas and percentages of cells stained by P16.

Table 7: Correlation between HPD and percentage of cells stained

			HPD			Total
			WDSCC	MDSCC	PDSCC	
Percentage of cells stained	Nil	No. of cases	26	10	2	38
		%	52%	20%	4%	76%
	1-10%	No. of cases	0	0	1	1
		%	0	0	2%	2%
	11-50%	No. of cases	8	1	0	9
		%	16%	2%	0	18%
	51-80%	No. of cases	1	0	0	1
		%	2%	0	0	2%
	81-100%	No. of cases	1	0	0	1
		%	2%	0	0	2%
Total		No. of cases	36	11	3	50
		%	72%	22%	6%	100%



Histopathological Images

Discussion

The most pivotal malignant tumor of the oral cavity is squamous cell carcinoma (SCC). Oral squamous cell carcinomas constitute 90% of oral cancers1. Oral squamous cell carcinoma (OSCC) is a carcinoma with squamous differentiation arising from the oral mucosal epithelium [1]. A total of fifty (50) biopsy and composite resected specimens of Oral squamous cell carcinomas received in the department of pathology, Sri Venkateswara medical college, Tirupati were studied.

Age-wise comparison: The mean age of presentation of oral squamous cell carcinoma is in the fifth and sixth decade of life in the population of Asia1. However, there is a steady increase in oral squamous cell carcinoma incidence in patients younger than 40 years. In our study majority of the OSCC patients were more than 50 years old (35 out of 50 cases), constituting 70% of the study. This is consistent with a study conducted by Tandon et al [6].

Sex wise comparison: Worldwide, oral squamous cell carcinomas are higher among males than

females. Males are more commonly affected than females by oral squamous cell carcinoma in both developed and developing countries which may be attributed to easy acceptance of habits by males. However, this gender distribution has been reducing due to an increase in females taking up tobacco-related habits like smoking. In our study of 50 OSCC, 27 (54%) were males, and 23 (46%) were females. Other studies conducted by Tandon et al[6]. 40 - males constitute 76.5% and females constitute 23.5%, MP Singh et al [6]. males constitute 75.9% and females constitute 24.1%.

Anatomical site-wise comparison: In India, 50% of oral cancers are reported from buccal mucosa. This may be due to betel quid, which is used to keep in the cheek pouch, directly contacting buccal mucosa. Therefore, the period of contact with tobacco products serves as a crucial factor in the causation of OSCC in a particular anatomic site. In the present study, anatomical site-wise majority of OSCC cases were from buccal mucosa, constituting 48% of the study (24 cases out of 50).This is followed by anterior 2/3 of tongue - 8 cases (16%), palate - 6 cases (12%), retromolar trigone - 4 cases (8%), gingival buccal sulcus - 3 cases (6%), maxillary alveolus - 3cases (6%), Mandibular alveolus - 1 case (2%) and lip -1 case (2%). In our study, OSCC arising from buccal mucosa out numbers OSCC from other sites, which is in concordance with the studies of Tandon et al [6].

Histopathology of oral squamous cell carcinoma: The main histological features of squamous cell carcinoma (SCC) are squamous differentiation and invasion. Squamous differentiation is characterized by keratin pearl formation and intercellular bridges. Invasion manifests as interruption of the basement membrane of surface epithelium, downward growth of the tumor tissue accompanied by the desmoplastic stromal reaction. Based on the degree of differentiation, cellular pleomorphism, and mitotic activity, SCCs are traditionally graded into three grades. Studies from all over India reported moderately differentiated squamous cell carcinoma (MDSCC) in most cases, probably due to the contribution of risk factors such as tobacco consumption. In the present study, most of the cases were well-differentiated squamous cell carcinoma, accounting for 72% (36 out of 50 OSCC cases), followed by 22% (11 out of 50 cases) of moderately differentiated squamous cell carcinoma and 6% (3 out of 50 cases) of poorly differentiated squamous cell carcinoma. Our study is in concordance with Tandon et al. (66.33% of WDSCC).

HPV association of oral squamous cell carcinoma: The oral cavity is lined by stratified keratinized and non-keratinized squamous epithelium, similar to the mucosal epithelium of the genital region. Both genital mucosal epithelium and oral epithelium are exposed to microorganisms, some of which may be potentially carcinogenic. The relevance of HPV infection in cervical cancers is well known. This consideration can imply that this link would also be present in relation to OSCC. HPV is a circular double-stranded DNA molecule, ~8kb, and over 100 genotypes have been reported. The HPV genome is composed of early genes that encode E1 to E7 proteins and late genes which encode L1 and L2 proteins.

Among these genes, E6 and E7 have significant functions in the malignant transformation of squamous cells. E6 binds to TP 53 and inactivates its function by ubiquitin-dependent degradation. E7 attaches to Retinoblastoma tumor suppressor protein (Rb) and activates transcription factor E2F, which enhances the expression of the cyclin-dependent kinase inhibitor 2A. There are more than twelve high-risk HPV. HPV 16 and 18 are more commonly detected in cervical and oropharyngeal squamous cell carcinomas. In India, there is variability in HPV (16 and 18) origin and progression in OSCC11. HPV infection in India estimates that 22.2% of OSCC are HPV associated. Patients with OSCC and HPV-positive have an improved prognosis compared to HPV-negative. These are also said to have lower recurrence rates after surgical removal of primary tumors. Hence it is essential to diagnose the HPV causation in OSCC. Some molecular techniques can be used for the detection of HPV, which include immunohistochemistry (using P 16) which is less sensitive and less specific, immunofluorescence, in situ hybridization, Southern blot test, dot blot test, and polymerase chain reaction (with high sensitivity). In addition, recent reports have suggested that overexpression of P16INK4A [7,8] protein expression can be used as a surrogate marker for diagnosing HPV-induced malignancies.

Our present study used P16INK4A (anti-human immunoglobulin) developed from mouse monoclonal antibodies. Our study showed 12/50 (24%), P16 positivity in Oral squamous cell carcinomas, and 38/50 (76%) P16 negativity. This comparison has a p-value of 0.45, which has less significance. The comparison is in concordance with studies of Singh RP et al [8]. Xia H et al [9]., Koppiker et al [10]., where P16 positivity were 6%, 2%, and 0%respectively.

Conclusion

The present study identified tobacco consumption as a significant risk factor in the majority of oral squamous cell carcinoma and its role as the most important etiological factor in the development of OSCC. In recent years, HPV has also emerged as a potential causative agent in OSCC. Using P16INK4A as a surrogate marker, the present study demonstrated HPV association in a small subset of cases (24%). This relatively low prevalence may be attributed to the limited sample size and the

restricted geographical area covered by the study. Therefore, studies involving larger sample sizes and broader geographic representation are needed to better clarify the relationship between HPV and OSCC. Furthermore, more sensitive HPV detection techniques, such as polymerase chain reaction (PCR), should be used alongside P16INK4A IHC to improve the accuracy of HPV detection and to better evaluate its role in the pathogenesis of OSCCs.

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