Original Article

Evaluation of P16 In Oral Cavity And Oropharyngeal Carcinoma

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ABSTRACT

Introduction: Oral cavity and oropharyngeal carcinoma is the sixth most common cancer worldwide, and squamous cell carcinoma is the most prevalent. The etiological factors are smoking, tobacco chewing, alcohol consumption, poor oral hygiene and human papilloma virus (HPV) infection. HPV infects basal epithelial cells through abrasion or minor trauma. Tumorigenesis is caused by HPV E6 and E7 proteins which decrease levels of p53 and Rb tumor suppressor proteins leading to aberrant overexpression of cell cycle protein p16.

Methods: This is a prospective, correlational cross-sectional study carried out on 46 cases of oral cavity and oropharyngeal carcinoma during the period of June 2021 to May 2022, at the Department of Pathology, Bir hospital and National Path Labs, Maharajgunj. The surgical biopsy cases were subjected to H&E staining and P16 immunohistochemistry.

Results: Total 46 cases of oral cavity and oropharyngeal carcinoma were enrolled in this study. The most common carcinoma was squamous cell carcinoma with a common age group between 41 to 60 years of age showing male predominance. Tongue was the most common site. Out of 46 cases, 34 cases were P16 positive and 12 were P16 negative. The correlation between the P16 immunohistochemistry and squamous cell carcinoma of oral cavity and oropharynx was significant ($p<0.005$).

Conclusion: There is a good correlation between P16 immunohistochemistry and squamous cell carcinoma of oral cavity and oropharynx and hence P16 can be used as a surrogate marker.

Keywords: *Oral cavity and oropharyngeal tumours, Squamous cell carcinoma, Immunohistochemistry, P16.*

Introduction

According to the GLOBOCAN (Global Cancer Incidence, Mortality and Prevalence) 2018, more than 800,000 new Head and Neck Squamous Cell Carcinoma cases are diagnosed annually worldwide comprising the sixth most common cancer leading to more than 400,000 deaths annually. Prevalence of oropharyngeal carcinoma in the world is 3.68 and in Nepal is 1.81 and of the oral cavity is 11.97 in the world and 8.23 in Nepal.[1]

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More than 90% of oral cavity and oropharyngeal carcinomas are squamous cell carcinoma.[2] The different etiological factors of oral and oropharyngeal squamous cell carcinoma are tobacco chewing, smoking, alcohol consumption, poor oral hygiene and Human papilloma virus. Human papillomavirus (HPV) is a small, non-enveloped DNA virus belonging to the Papillomaviridae family.[3]

HPV infects the basal epithelial cells through wounds and abrasions. The viral DNA then gets integrated into the host genome. Tumorigenesis is caused by HPV E6 and E7 proteins which decrease levels of p53 and Rb tumor suppressor proteins leading to aberrant overexpression of cell cycle protein p16.[3] P16 is a surrogate marker for the detection of HPV infection.[4]

As the etiology of HPV positive and negative oral and oropharyngeal carcinoma is different, their treatment and prognosis also differ. HPV-related cancer has a good

prognosis compared to non-HPV cases. So preventive measures in the form of HPV vaccination, targeted therapies against viral protein, and good response to treatment by HPV-positive cancers have made the need for pretreatment diagnosis of HPV-associated cancer and p16, a surrogate marker for the diagnosis.

Since oral and oropharyngeal carcinoma is in increasing trend due to human papilloma virus and there has been less research in our country, this study tends to correlate p16 immunohistochemistry with oral cavity and oropharyngeal carcinoma.

Methods

This was a prospective, cross-sectional study conducted in the department of Pathology, National Academy of Medical Sciences, Bir Hospital, Kathmandu, from June 2021 to May 2022. Ethical approval was obtained from Institutional Review Committee of National Academy of Medical Sciences. Patients who have histopathologically proven oral cavity and oropharyngeal carcinoma and who gave consent to take part in the study were enrolled in the study. Patients who refused for study, benign lesion of oral cavity and oropharynx, inadequate biopsy, autolysed specimen, patients who have undergone chemotherapy or radiotherapy before surgery/biopsy were excluded from the study. A total of 46 cases fulfilling the criteria were included in the study.

The surgical biopsies from the oral cavity and oropharynx were collected in formalin and subjected to routine tissue processing and rotary microtome to prepare sections from paraffin blocks. Staining with H & E was done according to the guidelines given in the Bancroft's Theory and Practice of Histological Techniques 7th edition.

Histopathological diagnosis was established first by morphology on the H&E stained slide and the tissue block was taken to National Path Lab, Maharajgunj road, Kathmandu to perform immunohistochemistry staining for p16. The H&E slides were reported by multiple pathologists. The IHC stained slides were analyzed for p16 immunoreactivity and staining intensity. A known p16 expressing cervical SCC case was used as the positive control and sections of normal tonsil used for negative controls with each run.

Results

During the study period between June 2021 to May 2022, a total of 4132 biopsy specimen were received in the Department of Pathology, Bir hospital, Mahaboudha, Kathmandu out of which 46 were carcinoma of oral cavity and oropharynx and were included in our study.

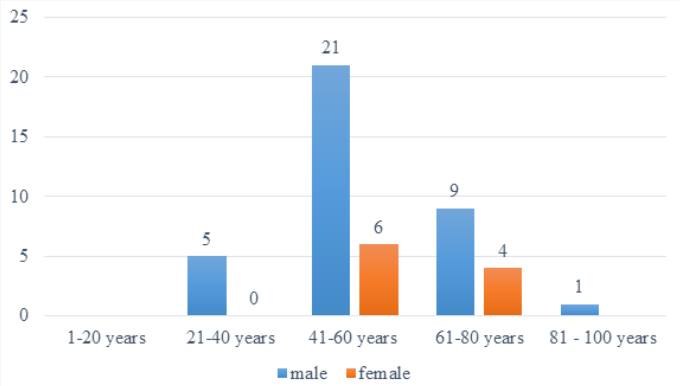

Chart 1: Incidence of Oral cavity and Oropharyngeal carcinoma with regard to age and sex.

Incidence of various oral cavity and oropharyngeal carcinoma in the present study is shown in Chart-1. Out of the 46 cases of oral cavity and oropharyngeal carcinoma, squamous cell carcinoma was the most common carcinoma constituting 97.80% (45/46) followed by adenocarcinoma (2.20%).

Table 1: Correlation of p16 immunoreactivity with subset of location of tumor

Site	P16 immunoreactivity		Total
	Positive	Negative	
Lip	1 (2.8%)	0	1
Tongue	14 (40%)	5	19
Gingivo buccal sulcus	2 (5.7%)	1	3
Buccal mucosa	5 (14.3%)	0	5
Tonsil	2 (4.3%)	0	2
Floor of mouth	4 (11.4%)	0	4
Base of the tongue	4 (11.4%)	2	6
Palate	2 (4.3%)	2	4
Retromolar trigone	1 (2.8%)	1	2
Total	35 (76.1%)	11 (23.9%)	46 (100%)

Table 1 demonstrates the correlation of different location of tumor with p16 immunoreactivity. This shows most common location of the tumor to be tongue i.e. 18 (39.1%) of the patient with maximum positivity. Lip, buccal mucosa, tonsil, floor of mouth and maxilla had 100% p16 immunoreactivity.

Table 2 shows 23.9% of the tumors were found to be negative immunoreactivity to P16 in which intensity score was"0". Whereas 15.2% of the tumor showed intensity score of 1+, 32.6% showed intensity score of 2+ and 28.3% of tumors showed maximum intensity score of 3+.

Table 2: Frequency of p16 intensity.

P16 immunoreactivity	P16 intensity	Frequency	Percent
Negative	0	11	23.9
Positive	1+	7	15.2
	2+	15	32.6
	3+	13	28.3
Total		46	100

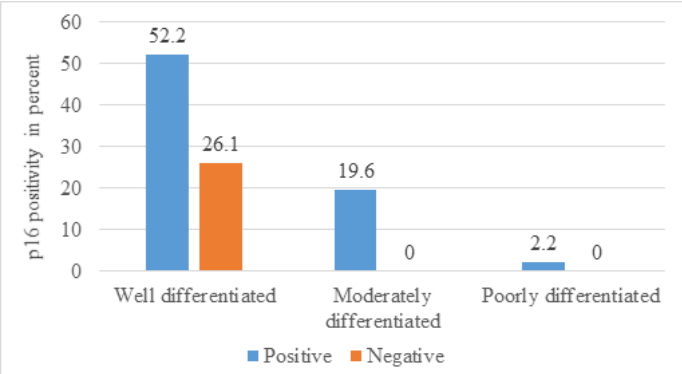

Chart 2: Correlation of p16 immunoreactivity with grading of the tumor.

Chart 2 demonstrates the correlation between tumor grades and p16 immunoreactivity. Well differentiated carcinoma constitute 78.3% in which 52.2 % are p16 positive and 26.1 % are negative while 19.6 % are moderately differentiated and 2.2 % are poorly differentiated both of which are 100% positive for p16.

Photomicrographs

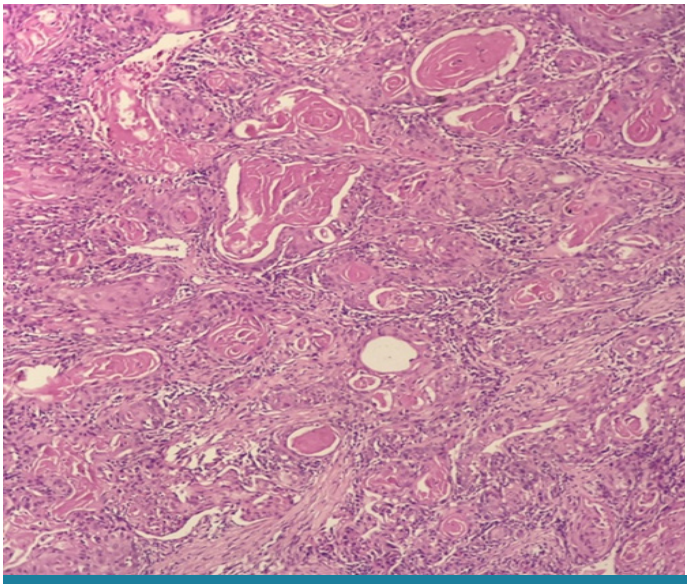

Fig 1: Well differentiated Squamous cell carcinoma. (Hematoxylene stain,100X)

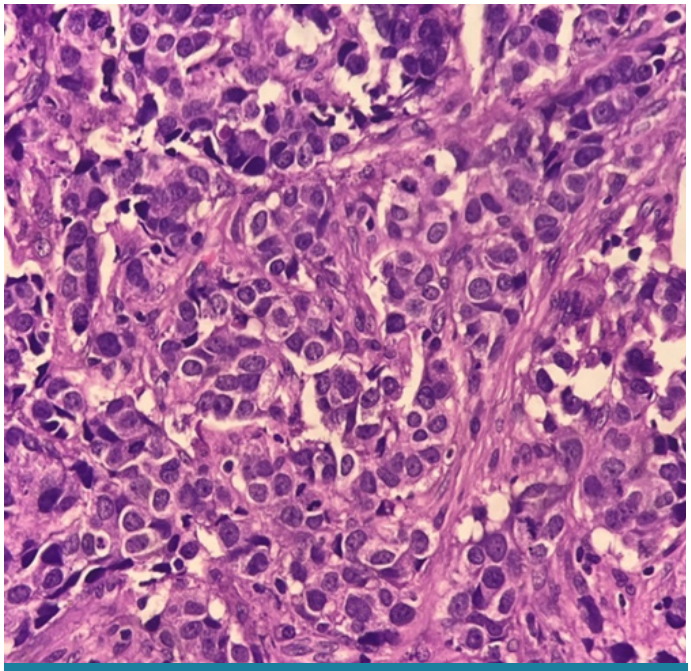

Fig 2: Moderately differentiated squamous cell carcinoma. (Hematoxylene stain, 400 X)

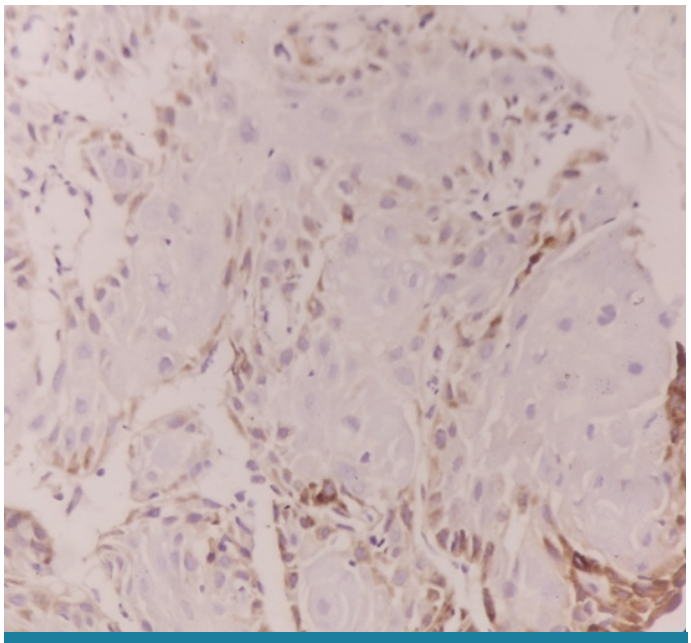

Fig 3: Picture showing focal P16 positivity with nucleus and cytoplasmic staining. (400 X) (P16 intensity score 1+)

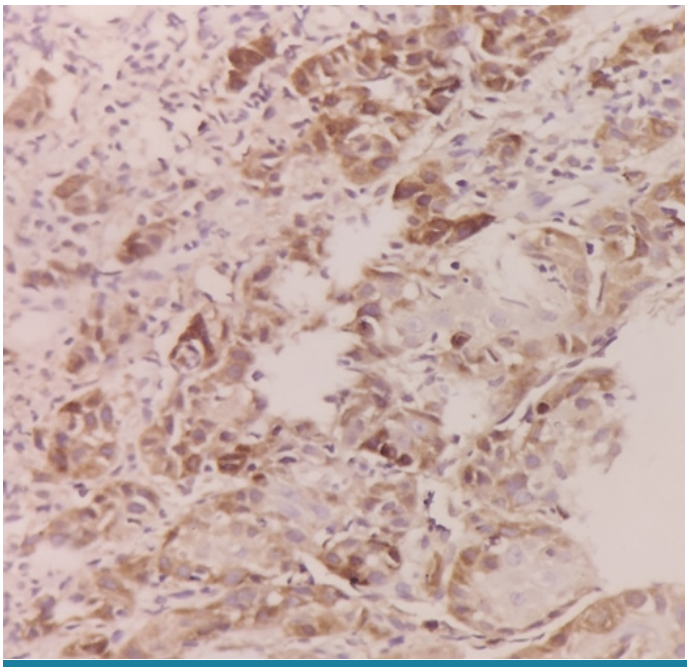

Fig 4: Picture showing diffuse nuclear and cytoplasmic staining of P16 in most of the area. (P16 intensity score 3+) (400 X)

Discussion

Squamous cell carcinoma is the most common carcinoma in oral cavity and oropharyngeal carcinoma.[5] Our study also show squamous cell carcinoma as the most prevalent carcinoma in the oral cavity and oropharynx which is 97.8% (45 out of 46 tumours in the oral cavity and oropharynx). One case was of adenocarcinoma of palate.

In the retrospective 10 year study done by Tandon et al. more than 90% oral cancers were squamous cell carcinoma arising from the mucous membrane of oral cavity and oropharynx.[6]

Our study shows that most oral cavity and oropharyngeal carcinoma fall under age group 41-60 years and there is a male predominance in all age group. Mean age of presentation is 56.4. Male constituted 78.2 % and female constituted 21.8% in overall age wise. The youngest age of presentation of the carcinoma is 33 years of age and the eldest age is 88 years of age.

In the study of Ghazawi et al. the incidence of oral cavity cancer was 50-69 years of age group while for oropharyngeal carcinoma it was 60-69 years of age group.[7]

A retrospective study done by Taylor et al. shows higher rate of squamous cell carcinoma in males than female for both HPV positive and HPV negative HNSCC.[8]

A retrospective study done by Lee et al. consisted of 1910 males (93.4%) and 136 females (6.6%). The male-to-female ratio was 14:1 in this study.[9]

Smoking and alcohol intake had no role in p16 status of oral cavity and oropharyngeal carcinoma in our study. Out of 46 cases in our study, 24 smokers were p16 positive and 7 smokers were p16 negative. Similarly, 11 non smokers were p16 positive and 4 non smokers were p16 negative. (P>0.05) hence, smoking was not significantly correlated with p16 status of the patient. Similarly, 26 out of 46 cases were alcoholic with 18 case p16 positive and 8 cases p16 negative. 20 cases were non alcoholic out of which 17 showed p16 immunoreactivity and 3 were p16 negative.

Allameh et al. have studied the relation between p16 status and various clinicopathological parameters including smoking. The relationship of smoking habit with p16 status in OSCC revealed that the difference in p16 expression in smoker and non-smoker patients was not correlated with p16 expression levels. There was a significant decrease in p16 gene expression in smoker patients compared to non-smoker patients ($p = 0.03$).[10]

Saito et al. in their study observed an increase in the prevalence of p16-positive OPSCC, nonsmoker OPSCC, and nondrinker OPSCC in Japan between 2000 and 2011. Multivariate analysis identified p16 expression and alcohol consumption as significant, independent prognostic markers of OPSCC.[11]

In our study 15 cases consumed tobacco and all were p16 immunoreactive however for non tobacco consumer 20 were p16 immunoreactive and 11 were p16 negative. The total number of patient who did not consumed tobacco was high (31 over 15) and p16 positivity was also high for them.

The study done by Murthy et al. showed high prevalence of tobacco use in oral cancers (83.5%). High prevalence of tobacco use was seen in both p16 positive and p16 negative carcinomas with more p16 positivity in non-user of tobacco.[12]

Tobacco and alcohol are still the etiological factors for oral cavity cancers, however their role in etiology of oropharyngeal carcinoma is in decreasing trend with human papilloma virus as the emerging etiological agent in the causation of oropharyngeal carcinoma.[13]

Our study showed 38 (82.6%) cases out of 46 were present in the oral cavity and 8 (17.4%) of 46 cases were present in the oropharynx. Tongue was the most common site of carcinoma in both the oral cavity and oropharynx with anterior 2/3rd of tongue for oral cavity and posterior 1/3rd of tongue for oropharynx. There was random distribution of p16 expression in different subset of oral cavity tumor.

Subset of oral cavity in our study included lip, tongue, gingivo buccal mucosa, buccal mucosa, floor of mouth, palate and retromolar trigone.

Squamous cell carcinoma (SCC) of the tongue is the most common oral cancer. Most cases occur on the lateral border of the tongue and only very rarely on the dorsum.[14]

A retrospective observational study done by Sharma et al. also found the most frequent oral cavity tumor to be tongue followed by buccal mucosa.[15]treatment, and outcomes of SCC of oral cavity and oropharyngeal cancer in the young age group of <40 years old.\nMATERIALS AND METHODS: This retrospective observational study was done by retriving data of selected cohort from 2013 to 2015. Patients were divided into 2 groups: Group 1 (10-30 years

A retrospective study done by Rikardsen et al. also showed mobile tongue as the most common site for oral squamous cell carcinoma.[16]

Our study showed good correlation of p16 with increasing grading of the tumor. 52.2% of the well differentiated carcinoma were p16 positive followed by 26.1% p16 negative. However, in moderately differentiated carcinoma and poorly differentiated carcinoma all the cases were p16 positive which comprised of 19.6% and 2.2 % of all the carcinoma respectively.

Grade is a strong and independent factor associated with distant metastasis in head and neck carcinomas. Thus, it adds important information to clinical and pathologic staging. It helps to identify patients at high risk for distant metastasis for whom an efficient systemic treatment is mandatory.

In the study of Ralli eta al., maximum number of cases belonged to Grade II (82.67%). Grade I and III tumors constituted 8% and 9.33% of cases, respectively. In their study, p16 expression had a significant correlation with histological grade of the tumor (P = 0.045). Out of 62 cases of Grade II (MDSCC), 25 (40.32%) cases showed strong p16 staining (Grade III), while only 11 (17.8%) cases were p16 negative. Out of 26 cases showing strong p16 staining (Grade III), maximum cases belonged to MDSCC category. Histological grade is a means of quantitating the degree of differentiation by applying a set of histological criteria. Usually welldifferentiated tumors are low grade lesions, whereas poorlydifferentiated tumors are high grade neoplasms.[17]

Conclusion

The most common cancer in oral cavity and oropharynx was squamous cell carcinoma. There was a male predominance in this type of tumor with male to female ratio of 3.5:1. The most common age group for the oral cavity and oropharyngeal carcinoma was between 41 years and 60 years. The common etiological factors for the carcinoma were tobacco chewing, smoking and alcoholism with human papilloma virus. The detection of HPV was done by demonstrating p16 immunohistochemistry. There was significant correlation between tumor, tumor grade and p16 immunohistochemistry (p<0.005%). Hence p16 immunohistohemistry can be used as independent marker for HPV detection in oral cavity and oropharyngeal carcinoma.

Recommendations

HPV status assessment in the form of P16 immunoreactivity may be helpful in early identification of cancer, determination of prognosis and posttreatment follow-up. HPV status should be included as an important risk and prognostic factor in future trials. Along with it, trials should be performed to get a proper treatment regime for HPV-positive and HPV-negative HNSCC in order to provide better treatment and lower relapse rates.

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