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Original Research Article

Evaluation of p16 as a Surrogate Marker in Squamous Cell Carcinoma of Oral and Oropharyngeal Regions

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ABSTRACT

Background
Squamous cell carcinoma (SCC) of the oral cavity and oropharynx is a prevalent malignancy often associated with lifestyle factors like tobacco and alcohol use, and increasingly, with Human Papillomavirus (HPV) infection. The p16 protein is widely used as a surrogate marker for HPV-associated SCC.

Objectives
To evaluate the expression of p16 in oral and oropharyngeal SCC, assess its correlation with histopathological grades, and explore its utility as a surrogate marker for HPV infection.

Methods
A prospective study was conducted on 50 patients diagnosed with oral and oropharyngeal SCC at NRI Institute of Medical Sciences, Visakhapatnam, from July 2018 to September 2020. Detailed histopathological grading and immunohistochemistry (IHC) for p16 expression were performed, and the results were analyzed statistically.

Results
Out of 50 cases, 12 (24%) were p16 positive. The most common site was the tongue (56%), followed by the buccal mucosa (26%). Well-differentiated SCC showed the highest p16 positivity (46%), and p16 positivity was predominantly associated with younger patients.

Conclusion
p16 serves as a promising surrogate marker for HPV in oral and oropharyngeal SCC, especially in well-differentiated tumors. This study highlights the potential clinical utility of p16 in the diagnosis and management of SCC. Further studies involving larger patient cohorts and molecular testing are recommended to validate these findings.
Keywords: Squamous Cell Carcinoma, p16, HPV, Oral Cancer, Oropharyngeal Cancer, Immunohistochemistry.

INTRODUCTION

Squamous cell carcinoma (SCC) of the oral cavity and oropharynx is one of the most prevalent head and neck malignancies, accounting for approximately 90% of oral malignancies worldwide. With an annual incidence of around 500,000 new cases, SCC poses a significant public health challenge, particularly in countries like India where oral cancer constitutes about 30% of all cancer cases.[1,2] Known risk factors for SCC include tobacco consumption, alcohol use, and betel quid chewing, which have historically contributed to its high prevalence.[3]

Human Papillomavirus (HPV), particularly HPV-16, has been recognized as a key etiological factor for a subset of oropharyngeal cancers. HPV-related SCC tends to occur in younger patients with fewer traditional risk factors, exhibiting distinct clinical and molecular characteristics, including p16 protein overexpression.[4,5] As a tumor suppressor protein, p16 is strongly expressed in HPV-positive tumors, where the viral oncoprotein E7 disrupts the Rb pathway, leading to uncontrolled cell proliferation.[6] Immunohistochemistry (IHC) for p16 has thus become a widely accepted surrogate marker for HPV infection in SCC.[7]

Understanding the role of p16 in SCC is crucial for guiding treatment decisions, as HPV-positive tumors are generally associated with better prognosis, higher response rates to radiation therapy, and improved overall survival compared to HPV-negative cancers.[8-10]

Given the rising incidence of HPV-related SCCs and the distinct clinical and prognostic implications, this study aims to evaluate the expression of p16 in oral and oropharyngeal SCC. Specifically, the study examines the correlation between p16 expression and histopathological grades of SCC, as well as its potential role as a reliable surrogate marker for HPV infection.

MATERIALS AND METHODS

Study Design

This prospective study was conducted at the Department of Pathology, NRI Institute of Medical Sciences, Visakhapatnam, over a two-year period from July 2018 to September 2020. The study included 50 patients diagnosed with SCC of the oral cavity and oropharynx. The inclusion criteria were histopathologically confirmed cases of SCC, while patients with other types of head and neck cancers were excluded.

Sample Collection and Histopathological Examination

Biopsy specimens were collected from all 50 patients and immediately fixed in 10% formalin. After fixation, the tissues were processed and embedded in paraffin. Sections of 5 micrometers thickness were cut and stained with hematoxylin and eosin (H&E) for histopathological

examination.[11] The tumors were graded based on the degree of differentiation into well-differentiated, moderately differentiated, and poorly differentiated SCC, according to the World Health Organization (WHO) classification.[12]

Immunohistochemistry for p16

Immunohistochemical staining for p16 was performed on formalin-fixed, paraffin-embedded tissue sections using a commercially available monoclonal antibody (CINtec® Histology Kit, Ventana).[13] The sections were deparaffinized, rehydrated, and subjected to antigen retrieval using a citrate buffer. After blocking endogenous peroxidase activity, the sections were incubated with the primary antibody for p16, followed by incubation with a biotinylated secondary antibody. The reaction was visualized using a DAB substrate and counterstained with hematoxylin.

A case was considered p16 positive if there was strong and diffuse nuclear and cytoplasmic staining in more than 70% of the tumor cells. Cases with weak or focal staining were considered negative.

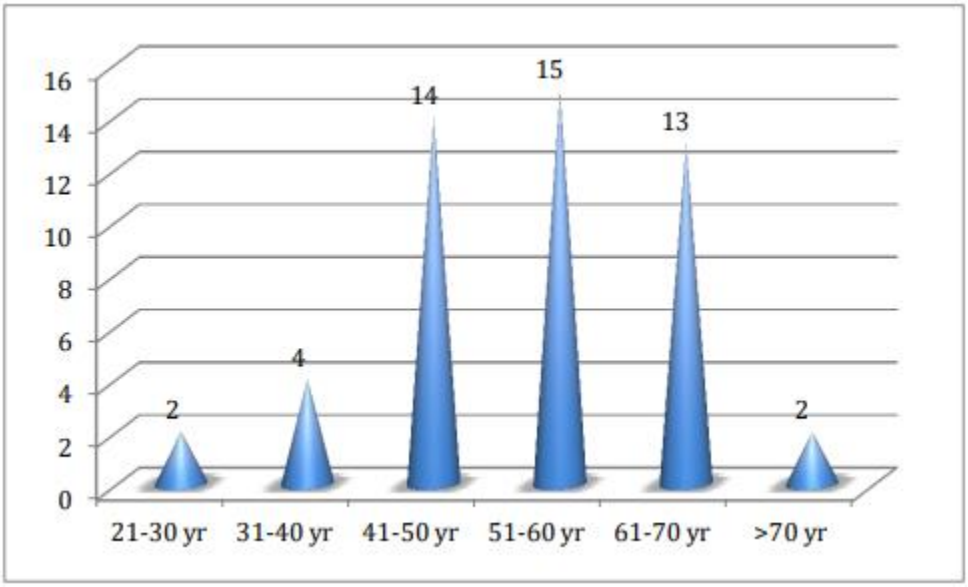
Statistical Analysis

The data were analyzed using SPSS software version 22.0. Descriptive statistics were used to summarize the demographic and clinical characteristics of the patients. The association between p16 expression and various clinical and histopathological parameters was evaluated using the Chi-square test. A p-value of less than 0.05 was considered statistically significant.

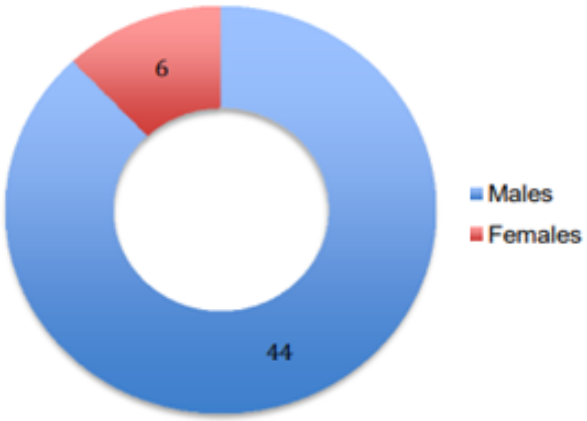
RESULTS

Demographic and Clinical Characteristics

The study cohort consisted of 50 patients with a mean age of 55 years (Graph 1). The majority of the patients were male (88%, n=44), with only 12% (n=6) being female (Graph 2). The most common site of SCC was the tongue, accounting for 56% (n=28) of cases, followed by the buccal mucosa (26%, n=13). Other sites included the hard palate (22%, n=11), tonsils (2%, n=1), and oropharynx (2%, n=1) (Graph 3).

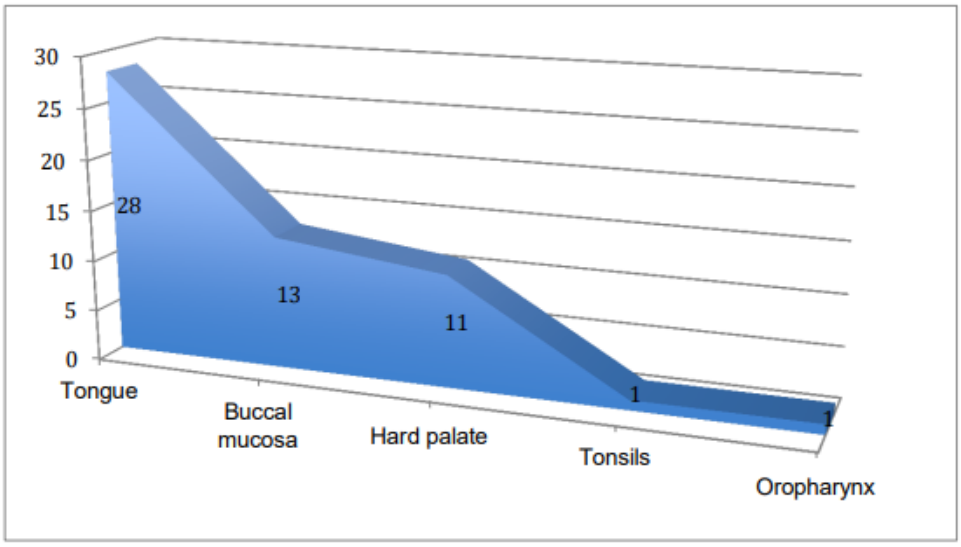


Graph 1: Age wise distribution of cases



Graph 2: Sex wise distribution of cases

In this study of 50 patients diagnosed with SCC, predominantly were males 44(88%) in number and the rest 6(12%) were females.



Graph 3: Cases according to location of the lesion

Histopathological Findings

Histopathological examination revealed that 46% (n=23) of the tumors were well-differentiated SCC (Figure 1), 42% (n=21) were moderately differentiated, and 12% (n=6) were poorly differentiated. The majority of the tumors were classified as the usual variant of SCC (84%, n=42), with a few cases of verrucous SCC (8%, n=4), and one case each of sarcomatoid, adenoid, and adenomatous SCC variants (2%, n=1 each).

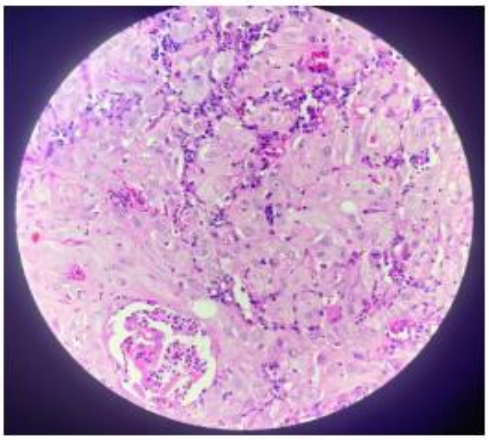


Figure 1: Well differentiated SCC 40X

p16 Expression

Immunohistochemical analysis showed that 24% (n=12) of the cases were p16 positive (Figure 2). Among the p16-positive cases, 75% (n=9) were classified as the usual variant of SCC. The distribution of p16 positivity across histological grades was as follows: 46% (n=6) in well-differentiated SCC, 42% (n=5) in moderately differentiated SCC, and 12% (n=1) in poorly differentiated SCC.

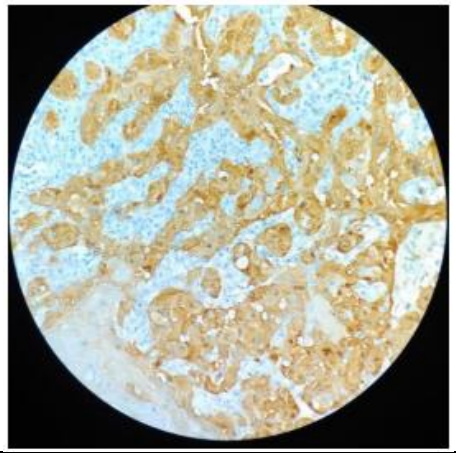


Figure 2: Positive P16 IHC Staining 40X

Statistical analysis revealed a significant correlation between p16 positivity and younger age ($p < 0.05$). p16 positivity was also more common in well-differentiated tumors and in tumors located on the tongue. No significant correlation was found between p16 expression and gender, tobacco use, or alcohol consumption.

DISCUSSION

The findings of this study are consistent with previous research highlighting the role of p16 as a surrogate marker for HPV in SCC of the oral cavity and oropharynx. In this study, 24% of cases showed p16 positivity, which aligns with reported rates ranging from 20% to 50% in similar studies.[14] The association of p16 positivity with well-differentiated SCC in younger patients further supports the hypothesis that HPV-related cancers tend to occur in individuals with fewer traditional risk factors like tobacco and alcohol use.[15] For example, Patel et al. reported that HPV-positive SCCs were more likely to present in younger patients and showed a favorable prognosis compared to HPV-negative tumors.[16]

Several studies have emphasized the prognostic implications of p16 positivity in head and neck cancers. Durazzo et al. reported that patients with p16-positive tumors had a higher survival rate and a better response to chemoradiotherapy than those with p16-negative tumors.[17] Similarly, Gillison et al. found that p16-positive oropharyngeal cancers were associated with improved outcomes, including better disease-free survival and overall survival, compared to HPV-negative tumors.[18] The present study supports these findings, as p16 positivity was predominantly observed in well-differentiated SCC, which is generally associated with better clinical outcomes.

The significant correlation between p16 positivity and tumors located on the tongue in this study echoes the findings of several other investigations. Iype et al. observed a similar distribution, with p16 positivity being more frequent in tongue SCC compared to other anatomical sites.[19] This site-specific association may reflect the higher susceptibility of the tongue's epithelium to HPV infection, further underscoring the importance of p16 as a diagnostic tool in oral and oropharyngeal cancers.[20]

In terms of histopathological grading, well-differentiated SCC showed the highest p16 positivity in this study, consistent with previous research. Brandizzi et al. noted that well-differentiated SCCs are more likely to be HPV-related and express p16, which may explain the better prognosis observed in these tumors.[21] This association between p16 positivity and tumor differentiation highlights the role of HPV in driving tumor biology and reinforces the prognostic significance of p16 testing in clinical practice.

However, it is important to acknowledge the limitations of using p16 as a sole marker for HPV infection. While p16 is a reliable surrogate marker, false positives can occur due to other mechanisms of p16 overexpression unrelated to HPV.[22] Studies like those by Syrjänen and Ringström have suggested that combining p16 testing with direct HPV DNA testing, such as polymerase chain reaction (PCR) or in situ hybridization (ISH), could enhance diagnostic accuracy and reduce the risk of misclassification.[23,24]

This study also observed a significant correlation between p16 positivity and younger age, consistent with the findings of Fakhry et al., who reported that HPV-related SCCs are more common in patients under 50 years of age.[25] In contrast, HPV-negative tumors tend to occur in older individuals with a history of tobacco and alcohol use, which was also observed in the present study.[26] The younger age of p16-positive patients in this cohort further supports the notion that HPV-associated SCC represents a distinct clinical entity with unique epidemiological and molecular characteristics.

Overall, this study adds to the growing body of evidence supporting the clinical utility of p16 as a surrogate marker for HPV in SCC of the oral cavity and oropharynx. However, the relatively small sample size and the absence of direct HPV testing are notable limitations. Future studies involving larger patient cohorts and molecular testing for HPV DNA are needed to validate these findings and further elucidate the role of p16 in predicting treatment outcomes and prognosis in SCC.[27]

CONCLUSION

This study concludes that p16 is a promising surrogate marker for HPV in SCC of the oral cavity and oropharynx. The significant association between p16 positivity and well-differentiated tumors, particularly in younger patients, underscores the potential of p16 in the clinical management of SCC. The findings of this study highlight the need for further research to validate the role of p16 in predicting treatment outcomes and improving the prognosis of patients with SCC.

LIMITATIONS OF THE STUDY

This study has several limitations, including the relatively small sample size and the lack of direct HPV DNA testing. While p16 is a reliable surrogate marker for HPV, it is not specific to HPV, and false positives can occur due to other mechanisms of p16 overexpression. Future studies should include molecular testing for HPV to confirm the findings of this study and further explore the relationship between p16 expression and clinical outcomes.

ETHICAL CLEARANCE

This study was approved by the Institutional Ethical Committee on September 22, 2018, with the letter number IEC/NRIIMS/2018/039

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