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Correlation of HPV-16, HPV-18 Genotypes with p16 Expression in Head and Neck Cancer: A Study from Western India

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Abstract

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Objectives This retrospective study aims to elucidate the clinical associations of HPV-16, HPV-18, and p16 expression with clinicopathological parameters, risk behaviors, and survival outcomes in head and neck cancer patients from the western Indian population.

Methods Clinical data of total 92 enrolled HNC patients diagnosed between years 2021 and 2023 were retrospectively collected from medical records. Formalin-fixed, paraffin-embedded blocks of all enrolled patients were collected whose p16 expression by immunohistochemistry tests were already performed. HPV-16 and HPV-18 infection was studied by real-time polymerase chain reaction. Associations between viral status, p16 expression, clinicopathological parameters, risk behaviors, and survival outcomes were assessed using SPSS statistical software version 20. *p*-Value $\leq$0.05 was considered to be statistically significant.

Results Among the 92 enrolled HNC patients, HPV-16 infection was detected in only 12 (13%) patients, with the remaining 80 (87%) testing negative. No HPV-18 infections were observed in any patient. Additionally, p16 expression was positive in only 13 (14%) patients, while 79 (86%) showed negative expression. A statistically significant correlation was found between metastasis involvement and positive HPV-16 infection ($p < 0.001$), with all HPV-16-positive cases exhibiting metastasis. A trend was also noted between the base of tongue and other clinical site subtypes with positive HPV-16 infection ($p = 0.063$). However, no other clinicopathological or risk behaviors showed significant associations with HPV-16 infection and p16 expression. Overall survival

Keywords

- clinicopathological parameters
- head and neck cancer
- HPV-16
- HPV-18
- p16 expression
- risk behaviors
- survival outcomes

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analysis revealed that neither HPV-16 infection nor p16 expression served as significant prognosticators in the HNC patient cohort ($p > 0.05$).

Conclusion This study provides comprehensive insights into the clinical relevance of HPV-16, HPV-18 infections, and p16 expression in HNC among the western Indian population. Understanding the associations between HPV-16, HPV-18, and p16 expression with clinicopathological parameters and survival outcomes may aid in optimizing patient management strategies, including personalized treatment approaches and targeted interventions. Further prospective studies are warranted to validate these findings and explore potential therapeutic implications.

Introduction

According to GLOBOCON 2020, head and neck cancer (HNC) poses a significant health burden, ranking as the seventh-most common malignancy globally.

In addition to traditional risk factors such as tobacco and alcohol consumption, emerging factors like genetic predisposition, dietary habits, and human papillomavirus (HPV) infection are gaining recognition as distinct contributors to HNC development. Epidemiological investigations in Western cohorts have illuminated a distinct rise in HPV-positive HNC cases, particularly in oropharyngeal squamous cell carcinoma. Among the plethora of HPV genotypes, HPV-16 and -18 are predominantly implicated in malignant alterations, with HPV-16 alone responsible for over 90% of HNSCC cases.[1] Persistent HPV infection triggers the overexpression of tumor suppressor protein p16, a biomarker often used for clinical detection of biologically active HPV infection. Moreover, the revised eighth American Joint Committee on Cancer/Union for International Cancer Control classification advocates for p16 as an HPV surrogate marker in Oropharyngeal Cancer (OPC) due to its correlation with enhanced survival rates.[2]

Against this backdrop, the present study aims to investigate the association of high-risk HPV-16, HPV-18, and p16 expression with clinicopathological parameters, risk behaviors, and survival outcomes in HNC among the western Indian population.

Materials and Methods

Patient and Sample Selection

This retrospective study involved 92 HNC patients diagnosed between years 2021 and 2023 at the Gujarat Cancer and Research Institute. It was approved by the Institutional Review Board and Ethics Committee. Formalin-fixed, paraffin-embedded (FFPE) blocks of primary tumor specimens were obtained from the archives of the Oncopathology Department of the institute. Histopathological details like cancer site, tumor differentiation, clinical stage, and p16 expression status were noted for all patients from the records of Oncopathology Department. Additional clinical details of all HNC patients like age, gender, occupation, demographic location, survival status, and history of tobacco exposure were archived from the Medical Record department.

HPV-16 and HPV-18 Detection by Real-Time Polymerase Chain Reaction

HPV-16 and HPV-18 detection was performed using real-time polymerase chain reaction (PCR). FFPE blocks of histologically confirmed HNC cases were utilized to obtain 9 to 10 µm sections (10 sections per case) in microcentrifuge tubes for DNA isolation. Genomic DNA extraction was performed using the QIAamp DNA FFPE Tissue Kit (Qiagen,), and the isolated DNA was quantified using the Qubit 3 Fluorometer.

DNA samples were detected for HPV-16 and HPV-18 expression using the commercially available HPV-16 and -18 detection kit through real-time PCR. The thermal profile included initial denaturation at 94°C for 10 minutes followed by 40 cycles of amplification and annealing at 94°C for 15 minutes and at 60°C for 1 minute. No amplification or amplification beyond 37 cycles was considered negative for HPV-16 or HPV-18.

Statistical Analysis

IBM SPSS statistical software version 20 was employed for statistical analysis. The chi-square test was utilized to assess the associations between HPV-16/-18 and p16 expression with clinicopathological parameters, tobacco exposure, geographic location, and occupation. Survival analysis was conducted using the Kaplan–Meier method and log-rank test. Statistical significance was determined at a p-value of ≤ 0.05.

Results

Prevalence of HPV-16, HPV-18 infection and p16 expression in HNC Patients

In this study, only 12/92 (13%) patients were positive for HPV-16 infection. Whereas, HPV-18 infection was not observed in any of the enrolled patients. Prevalence of p16 expression was observed only in 13/92 (14%) HNC patients.

Correlation of HPV-16 Infection with p16 Expression in HNC Patients

Through this study, no statistical significance was found to be associated with HPV-16 infection and p16 expression in the total 92 enrolled HNC patients. However, all 12 positive HPV-16 infection cases were negative for p16 expression and all 13 cases of positive p16 expression were associated with

absence of HPV-16 infection ($\chi^2 = 1.129$, $r = -0.157$, $p = 0.288$).

Correlation of HPV-16 and p16 Expression with Clinicopathological Parameters and Risk Behaviors in HNC Patients

In this study, the correlation between HPV-16 and p16 expression and clinicopathological parameters was explored among the 92 enrolled HNC patients. The findings indicate a statistically significant association between metastasis involvement and positive HPV-16 infection, as all cases of positive HPV-16 infection were accompanied by metastasis involvement ($\chi^2 = 13.800$, $r = +0.387$, $p < 0.001$). Additionally, a trend toward significance of a higher prevalence of HPV-16 infection was observed in patients with cancer in the base of tongue as compared with the patients with cancer in other sites of HNC ($\chi^2 = 3.455$, $r = +0.194$, $p = 0.063$) (Table 1). Moreover, HPV-16 infection and p16 expression were found to be associated with tobacco-habituated HNC patients. Conversely, no significant associations were identified between HPV-16 expression and other clinicopathological factors and risk behaviors. Further, no statistically significant correlation was observed between p16 expression and clinicopathological parameters or risk behaviors investigated in this study (►Table 1).

Association of HPV-16 Infection and p16 Expression with Overall Survival in HNC Patients

The relationship between HPV-16 infection, p16 expression, and overall survival (OS) in HNC patients was investigated in this study. A follow-up period of 18 months yielded complete follow-up details for 55 out of the initial 92 HNC patients, forming the cohort for OS analysis. Kaplan–Meier survival analysis was conducted to assess OS in these 55 patients, stratified by HPV-16 infection and p16 expression status.

The log-rank test was employed to compare survival curves. It was observed that the OS of HNC patients having HPV-16 infection was comparatively lower than the patients who did not have HPV-16 infection. However, HPV-16 infection did not emerge as a significant prognostic factor for OS in HNC patients (log rank = 0.963, df = 1, $p = 0.326$; ►Fig. 1A).

Similarly, p16 expression in HNC patients did not demonstrate prognostic capability for OS (log rank = 0.043, df = 1, $p = 0.835$; (►Fig. 1B). Furthermore, the combined analysis of HPV-16 infection and p16 expression status did not reveal any significant association with OS (log rank = 0.244, df = 1, $p = 0.622$; ►Fig. 1C).

Discussion

Increasingly, HPV infection is acknowledged as a significant contributor to carcinogenesis in various human cancers. High-risk HPV strains, notably HPV-16 and HPV-18, are implicated in the development of cancers across anatomical squamous tissue sites, including the head and neck regions. Many studies across the globe have reported that HPV prevalence exhibits considerable variation worldwide, ranging from 15 to 49%. This discrepancy is evident between developed and developing nations, with a larger proportion of HPV-positive HNC cases observed in developed regions. Moreover, Indian studies highlight wide geographical diversity in HPV prevalence, spanning from 0 to 74%.[3,4]

In the present study, HPV-16 infection was detected in 13% of HNC patients, while 87% patients tested negative for HPV-16 infection. Conversely, no instances of HPV-18 infection were identified among the enrolled HNC patients. These findings are consistent with previous studies conducted in North, West, and Central India, indicating similar patterns of HPV-16 and HPV-18 prevalence. However, it is noteworthy that HPV-18 exhibits higher prevalence rates in southern India.[1,4] Notably, current results strongly suggest that HPV-18 may not play a significant role in oral carcinogenesis in the studied population.

The current study showed that only 14% of HNC patients exhibited positive p16 expression, while 86% displayed negative p16 expression. However, this discrepancy diverges from findings in prior research that consistently highlighted the association between overexpression of p16 and HPV infection. Consequently, immunohistochemical expression of p16 is widely considered as a surrogate marker for oncogenic HPV infection.[5]

Furthermore, in the present study, all cases positive for HPV-16 had oropharyngeal cancer, while no cases of HPV-16 infection were detected in oral cavity cancers. These findings align with previous research indicating a significantly lower prevalence of HPV infection in oral cavity cancers compared with oropharyngeal cancer. Thus, the oral cavity appears to be relatively less susceptible to HPV carcinogenicity, although the underlying reasons for this anatomical site's differing vulnerability remain unresolved.[6] Additionally, in the present study, a trend of increased HPV-16 expression was observed in patients with cancer in the base of tongue in relation to patients with cancer in other clinical site subtypes ($p = 0.063$).

Statistically significant association was observed between positive HPV-16 infection and metastasis involvement, as all cases with HPV-16 infection had presence of metastasis ($p < 0.001$). Moreover, findings of the present study indicated that although not significant, HPV-16 infection tended to be more prevalent among younger age groups (≤59 years). This observation is consistent with a prior study,[1] which reported that HPV-16 positivity was more common in oral squamous cell carcinoma cases among younger individuals aged 21 to 44 years.

Additionally, all cases in this study exhibited advanced stages of HNC, with the majority reporting a history of prolonged and intense tobacco exposure, a recognized risk behavior. Further, it was observed that a majority of HNC patients with history of tobacco exposure exhibited positive HPV-16 and p16 expression. These findings contradict a previous study, which reported a significant overexpression of p16 in patients with HNSCC who did not have a history of tobacco exposure, particularly those who chewed paan.[5] Similarly, a population study in Brazil identified a co-occurrence of risk factors, namely tobacco and alcohol, alongside a low frequency of HPV DNA, leading to the conclusion that

Table 1 Correlation of clinicopathological parameters and risk behaviors with HPV-16 infection and p16 expression in HNC patients

		HPV-16 infection		p16 expression	
Characteristics	***N***	**Negative, *N* (%)**	**Positive, *N* (%)**	**Negative, *N* (%)**	**Positive, *N* (%)**
Age					
≤59 years	47	40 (85)	7 (15)	38 (81)	09 (19)
>59 years	45	40 (89)	5 (11)	41 (91)	04 (09)
		$\chi^2 = 0.290$, $r = +0.056$, $p = 0.595$		$\chi^2 = 1.994$, $r = -0.147$, $p = 0.161$	
Gender					
Male	83	73 (88)	10 (12)	72 (87)	11 (13)
Female	9	07 (78)	02 (22)	07 (78)	02 (22)
		$\chi^2 = 0.115$, $r = +0.090$, $p = 0.734$		$\chi^2 = 0.053$, $r = +0.076$ $p = 0.818$	
Clinical sites					
Oropharyngeal	86	74 (86)	12 (14)	75 (87)	11 (13)
Oral cavity	6	06 (100)	0 (0)	04 (68)	02 (33)
		$\chi^2 = 0.126$, $r = +0.102$, $p = 0.723$		$\chi^2 = 0.625$, $r = +0.146$, $p = 0.429$	
Clinical subtype					
Others	38	36 (95)	02 (05)	33 (87)	05 (13)
Base of tongue	54	44 (82)	10 (18)	46 (85)	08 (15)
		$\chi^2 = 3.455$, $r = +0.194$, $p = 0.063$		$\chi^2 = 0.050$ $r = +0.023$, $p = 0.825$	
Tumor differentiation					
Moderate differentiation	78	66 (85)	12 (15)	67 (86)	11 (14)
Poor differentiation	14	14 (100)	00 (00)	12 (86)	02 (14)
		$\chi^2 = 1.306$, $r = -0.164$, $p = 0.253$		$\chi^2 = 0.000$, $r = +0.002$, $p = 1.000$	
Lymph node involvement					
Absent	5	05 (100)	00 (00)	03 (60)	02 (40)
Present	87	75 (86)	12 (14)	76 (87)	11 (13)
		$\chi^2 = 0.043$, $r = +0.093$, $p = 0.835$		$\chi^2 = 2.916$, $r = -0.178$, $p = 0.088$	
Metastasis involvement					
Absent	46	46 (100)	00 (00)	37 (80)	09 (20)
Present	46	34 (74)	12 (26)	42 (91)	04 (09)
		$\chi^2 = 13.800$, $r = +0.387$, $\mathbf{p < 0.001}$		$\chi^2 = 2.240$, $r = -0.156$, $p = 0.138$	
Tobacco exposure					
Nonhabituated	4	04 (100)	00 (00)	03 (75)	01 (25)
Habituated	88	76 (86)	12 (14)	76 (86)	12 (14)
		$\chi^2 = 0.001$, $r = +0.083$, $p = 0.974$		$\chi^2 = 0.000$ $r = -0.067$, $p = 1.000$	
Geographic location					
Rural	58	51 (88)	07 (12)	50 (86)	08 (14)
Urban	34	29 (85)	05 (15)	29 (85)	05 (15)

Table 1 (*Continued*)

		HPV-16 infection		p16 expression	
Characteristics	***N***	**Negative, *N* (%)**	**Positive, *N* (%)**	**Negative, *N* (%)**	**Positive, *N* (%)**
		$\chi^2 = 0.002$, $r = +0.038$, $p = 0.967$		$\chi^2 = 0.000$ $r = +0.013$, $p = 1.000$	
Occupation					
Farmers/laborers	68	60 (88)	08 (12)	59 (87)	09 (13)
Others	24	20 (84)	04 (17)	20 (83)	04 (17)
		$\chi^2 = 0.068$, $r = +0.064$, $p = 0.794$		$\chi^2 = 0.005$ $r = +0.043$, $p = 0.941$	

Abbreviations: HNC, head and neck cancer; HPV, human papillomavirus.

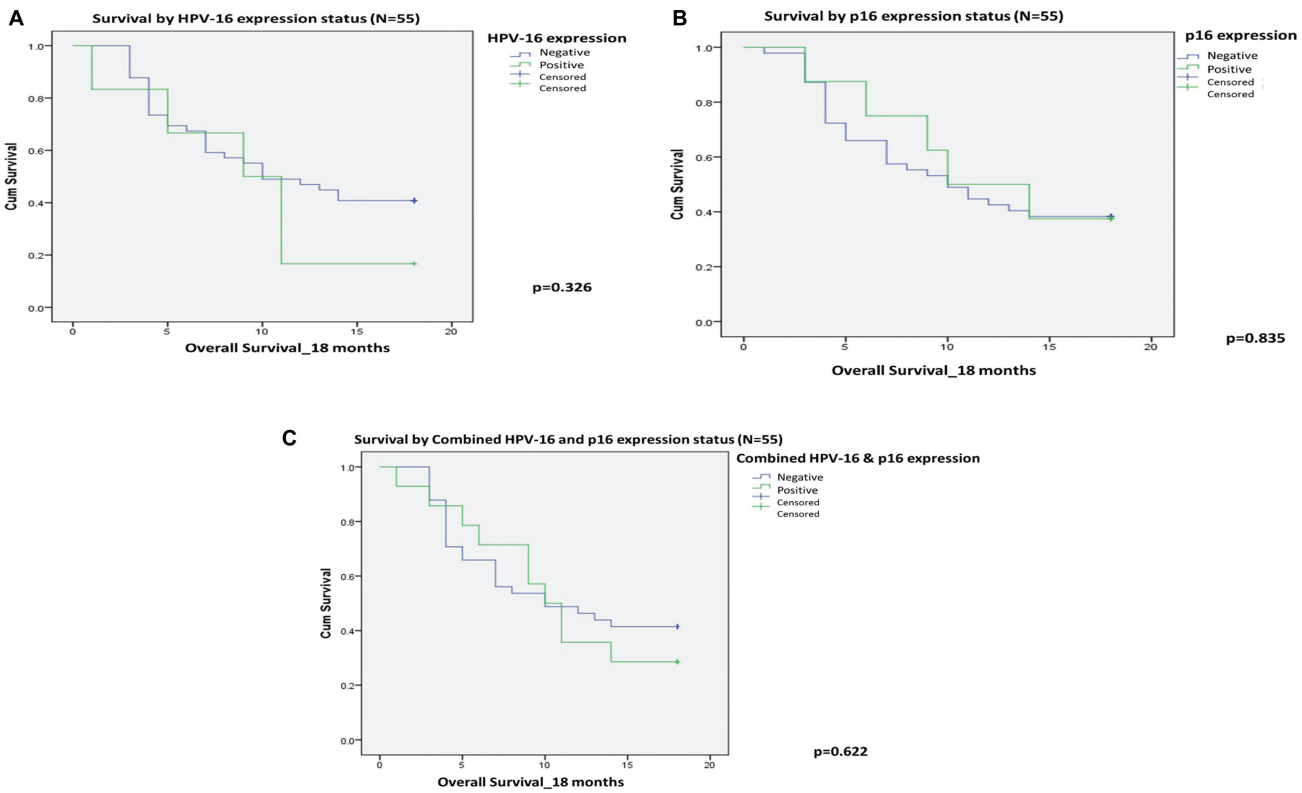

Fig. 1 Kaplan–Meier curves with log-rank analysis of overall survival. (**A**) Association of HPV-16 infection with overall survival. (**B**) Association of p16 expression with overall survival. (**C**) Association of combined HPV-16 and p16 expression with overall survival.

HPV may not play a significant role in the development of oral cavity squamous cell carcinoma.[6] Hence, future studies may necessitate robust strategies to explore HPV infection while considering major confounding factors.

Moreover, the current study did not reveal any significant correlations between p16 expression and clinicopathological factors or risk behaviors. This is consistent with prior research findings where no notable associations were observed between p16 expression and variables such as gender, age, tumor differentiation, lymph node involvement, or survival outcomes.[7] Similarly, there was no significant relationship observed between p16 expression and tumor site, which is in line with earlier studies that also found no statistical differences in p16 expression across various tumor sites.[5,8]

This study found that HPV-16 infection was not a significant predictor of OS in HNC patients. These results contradict the findings of prior studies in Western countries, where it was reported that patients with HPV-positive cancer exhibited better OS and progression-free survival compared with patients with HPV-negative cancer.[9,10] Nevertheless, our findings indicated that there was no significant correlation between p16 expression and OS, consistent with previous research findings.[5,11] The data presented in this study indicate that there is no significant correlation between HPV-16

infection and p16 expression. Additionally, a prior study has reported for prognostic evaluation, and p16 alone appears to be adequate, while direct detection of HPV-DNA should be incorporated for therapy stratification.[10]

Summarizing the findings from present study, a small number of HNC cases, primarily localized in the oropharyngeal region, attributed to HPV-16 infection. The expression of the p16 protein did not serve as an accurate surrogate marker for HPV-16 infection in HNC. Furthermore, HPV-16 DNA was detected in some HNC cases that were negative for p16 expression. Given the limited number of HPV-16 DNA-positive cases in our study, further investigation is warranted to explore the presence of HPV DNA and the expression of E6 and E7 mRNA, irrespective of p16 expression, using a larger cohort. It is important to note that our study had limitations, such as the utilization of samples from FFPE blocks instead of fresh tissue. Additionally, our cohort comprised tumors from various sites of HNC (oral cavity and oropharyngeal sites) treated with diverse regimens, which could potentially obscure significant findings.

Conclusion

In view of outcomes discussed, diverse observations related to HPV infection and p16 expression as compared with studies from Western countries may be attributed to high prevalence of tobacco exposure in the studied HNC patients from western India. This emphasizes the importance of understanding the associations between HPV-16, HPV-18, and p16 expression with clinicopathological parameters, risk behaviors, and survival outcomes among HNC patients. Furthermore, it may be suggested that comprehensive multi-institutional efforts are mandatory to gain a better understanding of oral carcinogenesis and factors that affect its clinical relevance in terms of staging and treatment strategies.

Source of Support

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Conflict of Interest

None declared.

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