ORIGINAL ARTICLE

Prevalence of *p16*-positive Squamous Cell Carcinoma of the Oropharynx in a Tertiary Government Hospital in the Philippines

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ABSTRACT

Objective. This study aimed to determine the hospital-based prevalence of human papilloma virus (HPV)-associated oropharyngeal squamous cell carcinoma (SCC) among patients in a tertiary government institution in the Philippines using immunohistochemical (IHC) staining for *p16* as surrogate marker.

Methods. This retrospective cross-sectional study included patients with oropharyngeal SCC from January 1, 2015 to December 31, 2021 who had available histopathology tissue blocks. Age, sex, and primary tumor site were extrapolated from charts and *p16* status was determined using immunohistochemistry staining. Chi-square test and Fisher's exact tests were performed for select variables and *p16* positivity. A p-value of <0.05 was deemed significant.

Results. Seventy-six patients were included, aged 32 to 84 years (mean: 61 years). Most were men (n=60, 78.9%), with male to female ratio of 3.75:1. The mean age of patients who were *p16*-positive were 5 years younger than *p16*-negative patients, although this was not found to be statistically significant (p=0.03, with Bonferroni Correction). Overall institutional *p16*-positive oropharyngeal SCC prevalence was 47.4% (n=36). More men (n=32, 88.9%) had *p16*-positive tumors compared to women (n=4, 11.1%), although sex only had a tendency for association with *p16* status (p=0.053). There was no significant difference between *p16* positivity across age groups. Majority with *p16*-positive results had tumors that were located in the tonsils (n=19, 52.8%). However, tumor subsite was not found to have statistically significant association with *p16* status.

Conclusion. The prevalence of HPV-associated oropharyngeal SCC was 47.37%, which was comparable to neighboring Asian countries – Japan and Taiwan. Multicenter studies with larger sample sizes may be explored for future investigations.

Keywords: p16, oropharynx, squamous cell carcinoma, HPV, human papilloma virus

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INTRODUCTION

Squamous cell carcinoma (SCC) of the head and neck has a documented global burden of more than 600,000 cases and more than 300,000 deaths.[1] The head and neck region spans different anatomic sites namely the sinonasal cavity, the nasopharynx, the oral cavity, the oropharynx, hypopharynx, larynx, and salivary glands. Taken altogether, malignancies in this region make it the sixth most common area of malignancy worldwide, with highest rates found in south and southeast Asian countries.[2] Histologically, around 90% of these head and neck malignancies are SCC.[3] Risk factors associated with several subtypes of head and neck SCCs include smoking or tobacco use, and alcohol consumption.[3] In addition to the two, the role of human papillomavirus (HPV) infection as a causative agent for head and neck SCC has been documented and has been found to be an independent prognostic factor specifically for oropharyngeal cancer (OPSCC).[4–7] Although oropharyngeal cancer ranks as the 6th most common head and neck cancer in the Philippines, HPV-associated oropharyngeal SCC infection has been found to be associated with increased survival and favorable outcomes.[8]

The NCCN Head and Neck Cancer version 5.2025 guidelines recommend evaluation of tumor HPV status by use of *p16* IHC as a surrogate marker of HPV status since it is a widely available biomarker that has shown a sensitivity range of 94-97% and specificity range of 83-84%.[9]

This study aimed to determine the hospital-based prevalence of *p16*-positive oropharyngeal SCC among patients in a Philippine tertiary government institution using *p16* immunohistochemical staining.

METHODS

A retrospective cross-sectional study including review of medical records and *p16* immunohistochemical (IHC) staining was performed. The study protocol was approved by the University of the Philippines Manila Research Ethics Board (UPMREB CODE: 2021-0203-01). The study was conducted from October 2021 to December 2022.

Inclusion and Exclusion Criteria

All registered patients with a histopathologic diagnosis of SCC of the oropharynx, who were admitted to the Department of Otolaryngology – Head and Neck Surgery (ORL-HNS) of the Philippine General Hospital were included in the study. In-patient as well as out-patient cases were included following availability of a registry maintained by the Department of ORL-HNS. From the list of identified patients with oropharyngeal cancer, patient information was cross-checked and verified with the records available with the Department of Pathology. Patients with non-SCC oropharyngeal malignancies, SCC from non-oropharyngeal sites, and patients without histopathologic tissue blocks for review were excluded. There were 152 identified patients with appropriate tumor location based from the ORL-HNS census. Of these, 32 were excluded due to histologic subtype not being established (incomplete IHC work-up, outside the scope of *p16* testing/non squamous cell carcinoma histology). Forty-four patients were excluded because the paraffin block was no longer available for *p16* IHC testing. Seventy-six remaining patients were included in the study.

Data Collection

Initial database search was done among admitted patients from January 1, 2015 to December 31, 2021. Data including age, sex, diagnosis, and primary tumor site were collected and checked for accuracy from the records of all patients in accordance with the Data Privacy Act of 2012. Patient records were assigned codes and anonymized.

p16 Status

Oropharyngeal subsites included in the study were the following: base of tongue, tonsil, soft palate, and oropharyngeal wall. *p16* immunohistochemical staining of the oropharyngeal SCC tumors was performed. *p16* expression was scored as positive if strong and diffuse nuclear and cytoplasmic staining were present in at least 70% of the tumor in accordance with the WHO essential criteria for HPV-associated oropharyngeal carcinoma.[10] The *p16* staining was carried out by two independent pathologists. Differing readings between the two pathologists were resolved via concurrence with a third pathologist.

Statistical analysis was done using SPSS ver. 28.0 (IBM, Chicago, Ill., USA). Chi-square test and Fisher's exact test for association, as well as analysis of variance were done. A p-value of <0.05 was deemed significant, using two-tailed comparisons for all analyses, except for the t-test for age and chi-square test for age groups for which Bonferroni correction was applied using the formula: $p \times n$ where p corresponds to the p-value of the analysis and n corresponds to the number of tests performed, in this case.[11]

Ethics Statement

The study protocol was approved by the Institutional Review Board of the University of the Philippines-Philippine General Hospital.

RESULTS

A total of 76 patients were included, aged 32 to 84 years (mean: 61 years). Most were men (n=60, 78.9%, with a male to female ratio of 3.75:1. Highest rates of oropharyngeal SCC patients were at the 61-70 age group (n=32, 42.1%). Overall *p16* prevalence in our sample was 47.4% (n=36). The mean age of patients who were *p16*-positive were 5 years younger than *p16*-negative patients, although this was not found to be statistically significant (p=0.03, with Bonferroni Correction). Fisher's exact test of association was done for *p16* status and sex, as one cell had n<5. *P16* positivity is more

common in men compared to women (88.9%, n=32 versus 11.1%, n=4), an association that showed tendency towards statistical significance (Fisher's exact test p value = 0.053). This Fisher's exact test may, however, lacked sufficient power due to large imbalance in the number of males and females (60 vs 16). There was no significant difference between *p16* positivity across age groups. Majority of participants with positive *p16* results had tumors that were located in the tonsils (n=19, 52.8%). Tumor site was not found to have statistically significant association with *p16* status. Analysis of variance showed no significant differences in *p16* positivity across age groups and tumor subsites. Although there was a trend showing that more than half of patients with tonsils affected had positive *p16* results, this association was not statistically significant (p=0.10). A summary of clinicopathologic data is found in Table 1.

DISCUSSION

HPV-associated squamous cell carcinomas of the head and neck are distinct groups of tumors with different malignant pathways, and pathologic as well as clinical features.[5] Molecular and serologic markers for HPV have been found in these subsets of head and neck SCCs detected by polymerase chain reaction (PCR) and in-situ hybridizations. Though PCR-based assays confer higher sensitivity, these are deemed labor-intensive, and costly.[7]

Overexpression of the *p16* immunologic marker has been shown to be a good surrogate marker for HPV infection.[8,12–14] Although the gold standard is the use of PCR for detection of E6/E7 mRNA for HPV, *p16* IHC has been shown to be a good surrogate for HPV since signaling of viral oncoprotein E7 induces overexpression of *p16* in HPV-associated cells or tissues.[15]

Recent scientific data suggest that prognosis from HPV-associated oropharyngeal SCC is more favorable than their non-HPV-associated counterparts.[4–7] In 2017, the American Joint Committee on Cancer (AJCC) released a new staging system for Oropharyngeal SCC in the 8th edition of the Cancer Staging manual specifically for this reason.[16] Compared to the previous edition, patients with HPV associated SCC of the oropharynx were down-staged to reflect the overall better prognosis of this specific subgroup.[17] The improved prognosis for patients with HPV-associated OPSCC relative to HPV-negative OPSCC is hypothesized to be partly due to the former's better response to chemotherapy and radiotherapy.[4]

There is limited regional data and Philippine data documenting the local prevalence of *p16* in oropharyngeal SCC.[18] In 2022, a hospital-based prevalence study from the Rizal Medical Center documented *p16* positivity across different head and neck subsites. The study included 24 patients with SCC in the larynx, oropharynx, and oral cavity. Only three patients with oropharyngeal SCC were included, two of which were *p16* positive. Overall *p16* positivity in their study, which included several subsites of the head and neck region was only 12%.[19]

In our sample, the hospital-based prevalence of HPV-associated oropharyngeal SCC based on *p16* IHC staining was 47.37%. This was comparable to the 41.5% prevalence in Japan, but was lower compared to a prevalence of around 70% documented in Korea and the United States of America.[5,6,20–22] A lower prevalence of 28.2% and 31% for

Table 1. Association of *p16* Positivity across Select Characteristics

Characteristics	Total, n (%)	*p16* Findings, n (%)		*p*-value
		Negative	Positive	
Total n (%)	76 (100%)	40 (52.63%)	36 (47.37%)	–
Age [mean (SD)]	61 (10.8)	63 (11.2)	58 (9.6)	0.06
Sex				0.05
Female	16 (21.05%)	12 (30%)	4 (11.11%)	
Male	60 (78.95%)	28 (70%)	32 (88.89%)	
Age Groups (years)				0.60
31 to 40	2 (2.63%)	1 (2.50%)	1 (2.78%)	
41 to 50	11 (14.47%)	5 (12.50%)	6 (16.67%)	
51 to 60	19 (25%)	8 (20%)	11 (30.56%)	
61 to 70	32 (42.11%)	16 (40%)	16 (44.44%)	
71 to 80	10 (13.16%)	8 (20%)	2 (5.56%)	
>80	2 (2.63%)	2 (5%)	0 (0%)	
Site affected				0.18
Base of tongue	18 (23.68%)	13 (32.50%)	5 (13.89%)	
Tonsil	32 (42.11%)	13 (32.50%)	19 (52.78%)	
Soft palate	7 (9.21%)	3 (7.50%)	4 (11.11%)	
Oropharyngeal wall	1 (1.32%)	1 (2.50%)	0 (0%)	
Not specified	18 (23.68%)	10 (25%)	8 (22.22%)	

SD – standard deviation

p16 in oropharyngeal SCC was determined for Thailand and Taiwan , respectively.[23,24]

There are limited available data on the prevalence and outcomes of HPV-related oropharyngeal SCC in the Philippines. This study was limited by its retrospective, single-center design with limited sample size. These factors may have contributed to selection bias, as only patients with complete data and available tissue samples for *p16* IHC processing were included. Longitudinal and multi-institutional studies in the future can be done to validate or compare with the positivity rate and demographic data obtained from this sample and provide more insight about this disease in the Philippine setting.

CONCLUSION

The hospital-based prevalence of *p16* positivity rate of oropharyngeal SCC in our sample was determined to be 47.37%. There were no significant differences in age between *p16*-positive and *p16*-negative patients, and no significant associations were observed between *p16* status and sex, age groups, or sites affected. This study provides an important reference for the *p16* positivity rate detected in a Philippine national tertiary referral center.

Statement of Authorship

All authors certified fulfillment of ICMJE authorship criteria.

Author Disclosure

All authors declared no conflicts of interest.

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