Prevalence of p16 expression in oropharyngeal squamous cell carcinoma in southern Brazil

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Objective. Our aim was to evaluate the prevalence of human papillomavirus (HPV)−positive tumors in a cohort of patients with oropharyngeal squamous cell carcinoma (OPSCC) at a single center in southern Brazil and determine the short-term prognostic factors in this sample.

Study Design. Ninety-one consecutive patients with newly diagnosed primary OPSCC between January 2017 and December 2019 were retrospectively included. Demographic, clinical, pathologic, and survival data were collected. HPV status was determined by using p16 immunohistochemistry.

Results. The overall prevalence of HPV-positive (HPV+) OPSCC was 20.9%. Patients with HPV+ tumors presented a nodal metastasis as the first clinical sign ($P = .02$); reported less alcohol ($P < .001$) and tobacco use ($P < .001$); exhibited lower tumor stages ($P < .001$) and higher microscopic grades ($P = .01$); and had higher chances of having resectable tumors ($P = .008$). p16-negative status ($P = .01$); unresectable/inoperable tumors ($P < .001$); presence of nodal metastasis ($P = .005$); and higher American Joint Committee on Cancer (AJCC) stage ($P = .002$) were significantly associated with worse disease-specific survival.

Conclusions. The prevalence of HPV+ OPSCC in southern Brazil is relatively low, and p16-positive status was associated with Better prognosis. Higher AJCC stage, nodal metastasis, and unresectability/inoperability were associated with the highest hazard ratios for death resulting from OPSCC. (Oral Surg Oral Med Oral Pathol Oral Radiol 2020;130:681−691)

The incidence rates of oral cavity cancer (OC) and oropharyngeal cancer (OPC) have remained stable or even declined since the 1980s, probably because of reductions in smoking and alcohol consumption,[1-3] a trend that is occurring in some underdeveloped countries. OC and OPC together rank as the fifth most common cancer in men in Brazil today.[4] Moreover, in Latin America, Brazil has the highest mortality rates for these malignancies, showing an increase since the 1980s. Only recently, Perea et al. showed stabilization in OC mortality rates and a decrease in OPC mortality rates.[5] Despite the overall downward trend in the incidence of OC and OPC at the end of the last century, since the 1990s the incidence of OPC has increased, particularly among relatively young individuals, men, and whites.[6,7] This increase in the number of new cases is already being observed in many geographic regions, such as the United States,[8] northern Europe,[9-11] Australia,[12] and Taiwan.[13] Squamous cell carcinoma (SCC) accounts for greater than 95% of the OPC cases, and human papillomavirus (HPV) is considered the cause of the rising incidence of OPC in those countries, where a gradual increase in the prevalence of HPV-positive (HPV+) oropharyngeal squamous cell carcinoma (OPSCC) has been observed.[6,7,9,12] Interestingly, patients with HPV-driven OPSCC generally demonstrate better survival rates compared with patients with HPV-negative (HPV−) OPSCC, independent of which treatment protocol is chosen.[14-17] Moreover, p16 has been demonstrated to be an independent surrogate marker, leading the last edition of the *AJCC Cancer Staging Manual* to incorporate p16 as a means to categorize OPC cases into those that are associated with high-risk HPV infection and those that are not. Consequently, therapy de-escalation for patients with HPV-associated OPSCC is currently being discussed and has given rise to the implementation of new clinical trials.[18]

The epidemiologic situation of HPV-related cancer in Brazil is still not very well explored.[19] A systematic review trying to consolidate the data regarding HPV-associated OC and OPC from published data in Brazil found a 27.4% (301 of 1097) prevalence of HPV+ tumors, although the majority of the studies were small and used nonprobability samples, not necessarily enrolling consecutive patients.[20] No studies from the state of Rio Grande do Sul were included. Recently, a multicentric study from São Paulo retrospectively evaluated 215 OPSCC cases,[21] and although it was not clear if the

Statement of Clinical Relevance

In southern Brazil, the prevalence rate of human papillomavirus−positive oropharyngeal squamous cell carcinoma (OPSCC), based on p16 immunohistochemistry testing, is relatively low. The current analysis showed a favorable prognosis for patients with p16-positive OPSCC compared with p16-negative cases.

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cases were consecutively collected, a high prevalence (59.1%) of HPV-related OPSCC was found, suggesting an increase in the incidence of HPV-related OPSCC over the last decade in Brazil. To further complicate matters, HPV status was not associated with higher survival rates. The only study in the south of Brazil was conducted in Curitiba, Paraná, where the authors found that 86.3% of tumors were p16 positive (p16+) among a series of 78 cases of OSCC, collected from 2005 to 2009.[22] Nevertheless, it is not clear if this sample enrolled consecutive patients, and a disparity was observed between these data and the results obtained in other Brazilian centers during a similar period.

At our institution, OPSCC has been the most prevalent head and neck cancer (HNC) since 2012, when there was a reversal of incidence trend between OC and OPC. We currently have a ratio of 3:2 new diagnoses of OPC compared with OC for each year (nonpublished data). Our hypothesis is that HPV-related cases might be influencing the high incidence of OPSCC seen at our institution. Therefore, the aim of this study is to evaluate the prevalence of p16+ status in a retrospective cohort of patients with OPSCC from a single center in southern Brazil and determine if HPV status influences the prognosis of this population.

MATERIALS AND METHODS

Study population

This study was approved by a regional Research Ethics Board Committee (No. 3.550.735). This study was based on a retrospective cohort of 91 consecutive patients with newly diagnosed primary OPSCC treated at the Department of Head and Neck Surgery at our hospital during a 3-year period, between January 2017 and December 2019. Our institution is the hub of cancer care for a micro-region of 33 municipalities, totaling around 750,000 inhabitants, approximately 6% of the state's population. Patients were identified from the Institution Cancer Registry by using the *International Classification of Diseases*, 10th edition (ICD-10) codes C01, C051, C052, C09, and C10 and also from operating theater and consultant records. All cases were reviewed by one of the head and neck surgeons from our service (F.M.G.), and patients with tumors that were not OPSCC were excluded. In patients with advanced tumors that had signs of invasion of other parts of the pharynx or of the oral cavity, an oropharyngeal primary tumor was defined on the basis of the tumor epicenter, history, and characteristics, as identified on clinical examination by a head and neck surgeon. The following data from patients' clinical records and their demographic characteristics were reviewed: smoking status, alcohol abuse (defined by at least 1 drink per day for women and at least 2 drinks per day for men),[23] tumor characteristics and staging, treatment information, and follow-up data (presence and date of recurrence, disease-specific survival [DSS] and overall survival [OS]). Tumor stage was based on the *AJCC Cancer Staging Manual*, 8th edition. Survival was defined as the time from the date of histologic proof of the OPSCC to death.

HPV status determination

Since January 2017, at our institution, all OPSCC cases are tested for the p16 marker. In cases where no information about p16 status was available, archived formalin-fixed, paraffin-embedded tissue blocks of OPSCC were retrieved from the regional laboratories for testing. p16 immunohistochemistry (IHC) staining was done routinely in 79 patients during investigations and retrospectively for 8 patients. In 4 patients with biopsy done outside our department, we could not get information regarding their p16 status. Those patients were excluded from the comparative analysis between p16+ and p16-negative (p16−) cases. IHC staining was performed on OPSCC formalin-fixed, paraffin-embedded 4 mm-thick sections by using a monoclonal antibody to p16 (CINtec Histology kit, Clone E6 H4) with a BondTMRX autostainer (Leica, Nuss-loch, Germany), according to the manufacturer's instructions. A known p16+ OPSCC sample was used as a positive control. Tumors showing 75% or greater diffuse p16 staining of cells, with at least moderate (+2 or +3) staining intensity, were deemed positive for HPV.

Statistical analysis

A descriptive analysis was performed for demographic, clinical, and pathologic features. Differences in HPV+ and HPV− cases were assessed through Fisher's exact test or the χ^2 test (for categorical variables), Student *t* test (for age), and the Mann-Whitney test (for the duration of symptoms). A univariate Cox regression model was used to evaluate the prognostic value of independent variables in determining OPSCC DSS. Then, a multivariate model combining p16 and AJCC stages was created to determine if HPV status remained a significant prognostic factor independent of clinical stage. Kaplan-Meier survival curves were constructed and compared by using the log-rank test. Data were analyzed by using SPSS software version 20 (SPSS Inc., Chicago, IL).The level of statistical significance was set at $P < .05$.

RESULTS

Sample characterization

The patient and tumor characteristics of the 91 patients diagnosed during 2017−2019 at our institution are shown in Table I. Men outnumbered women by a factor of 6. A large proportion of patients reported current or previous habit of smoking tobacco (n = 81; 89%) or

Table I. Demographic, clinical, and pathologic features of patients with OPSCC

	n = 91 (100%)
Gender, n (%)	
Male	78 (85.7%)
Female	13 (14.3%)
Age, in y	
Mean ± SD	61.28 ± 8.67
Range	42−84
Skin color, n (%)	
Caucasian	87 (95.6%)
Non-Caucasian	4 (4.4%)
Educational level	
Primary school	76 (83.5%)
High school	8 (8.8%)
Bachelor's degree	7 (7.7%)
Smoking status, n (%)	
Yes/former user	81 (89%)
No	10 (11%)
Alcohol use, n (%)	
Yes/former user	70 (76.9%)
No	20 (22%)
Missing data	1 (1.1%)
Histologic grade, n (%)	
Well-differentiated	20 (22%)
Moderately differentiated	54 (59.3%)
Poorly differentiated	9 (9.9%)
Missing data	8 (8.8%)
Initial symptoms, n (%)	
Upper aerodigestive tract	62 (68.1%)
Adenopathy	25 (27.5%)
No symptoms/casual diagnosis	4 (4.4%)
Site, n (%)	
Lateral pharyngeal walls	45 (49.5%)
Soft palate/uvula	16 (17.6%)
Posterior pharyngeal walls	4 (4.4%)
Base of the tongue/vallecula	25 (27.5%)
Missing data	1 (1.1%)
Duration of symptoms, in months	
Mean ± SD	6.16 ± 5.88
Range	0.5−24.0
Missing data	20 (22%)
Initial oral findings, n (%)	
Suspicious lesion	87 (95.6%)
Unsuspicious lesion	4 (4.4%)
Initial cervical findings, n (%)	
Suspicious lesion	68 (74.7%)
Unsuspicious lesion	23 (25.3%)
Resectability/operability, n (%)	
Unresectable/inoperable	35 (38.5%)
Resectable	56 (61.5%)
T, n (%)	
T1	18 (19.8%)
T2	15 (16.5%)
T3	24 (26.4%)
T4	34 (37.4%)
N, n (%)	
cN/pN 0	20 (22%)
cN/pN +	71 (78%)
TNM, n (%)	
x	4 (4.4%)
I	12 (13.2%)
II	10 (11%)
III	9 (9.9%)
IV	56 (61.5%)
Treatment, n (%)	
Surgery (with or without RT/CT)	25 (27.5%)
Radiotherapy (with or without SX/CT)	45 (49.5%)
Not performed	4 (4.4%)
Incomplete	17 (18.7%)
Metachronous or synchronous tumor, n (%)	
Present	16 (17.6%)
Absent	75 (82.4%)
Outcome, n (%)	
Alive	43 (47.3%)
Death caused by the tumor	44 (48.4%)
Lost to follow-up	4 (4.4%)
HPV status based on p16, n (%)	
Positive	19 (20.9%)
Negative	68 (74.7%)
Missing data	4 (4.4%)

CT, chemotherapy; *HPV,* human papillomavirus; *OPSCC,* oropharyngeal squamous cell carcinoma; *SD,* standard deviation; *RT,* Radiotherapy; *SX*, Surgery; *TNM,* tumor−node−metastasis.

current or previous alcohol abuse (n = 70; 76.9%). Most patients were diagnosed with an advanced disease stage: AJCC stages III and IV were found in 9 (9.9%) and 56 (61.5%) patients, respectively. Other demographic, clinical, and pathologic features are detailed in Table I.

Association of demographic, clinical, and pathologic features and HPV status

The prevalence rate of HPV+ OPSCC was 20.9%, based on the 87 cases tested for HPV (Figure 1). In 2017, 2018, and 2019, the prevalence of HPV+ cases was 21.1%, 31.6%, and 32.4%, respectively. Compared with patients with HPV− OPSCC, those with HPV+ tumors reported less alcohol use (38.9% vs 88.2%; $P < .001$)

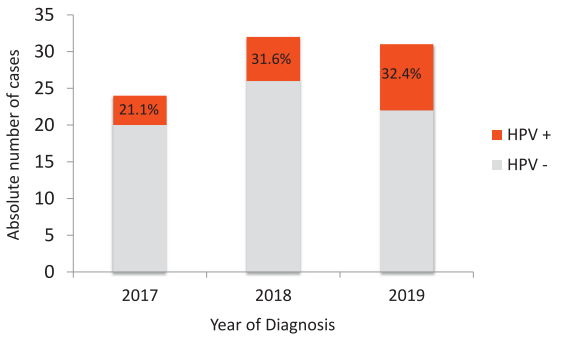

Fig. 1. Absolute number of human papillomavirus (HPV)−negative and HPV-positive cases according to year of diagnosis. The relative percentage of HPV-positive cases is detailed for each year.

and tobacco use (57.9% vs 97.1%; $P < .001$) and exhibited earlier tumor stages (stages I/II in 63.2% vs 14.7%, $P < .001$) (Table II). Moreover, significant differences were observed in initial symptoms, histologic grade, T stage, and resectability/operability of tumors, with a higher proportion of HPV+ cases presenting with neck nodes as the first symptom at clinical presentation ($P = .02$), small primary tumors ($P = .04$), poorly differentiated grade ($P = .01$), and higher chances of being resectable/operable ($P = .008$). There were no significant differences in age (average age 60.8 years vs 61.6 years, in p16+ vs p16− cases, respectively) and cN/pN status (N0 in 21.1% vs 22.1%, in p16+ vs p16− cases, respectively) according to p16 status. A tendency for a higher rate among women in the p16+ group was observed (26.3% vs 10.3%, in p16+ vs p16− cases, respectively), although without statistical significance ($P = .08$). There was no significant differences in skin color, duration of symptoms, type of treatment, and presence of synchronous metastasis with regard to HPV status either ($P > .05$). Topographically, HPV+ and HPV− tumors were most frequently located in the tonsils and at the base of the tongue (see Table II).

Survival analysis

Only patients treated with curative intent were included in the survival analysis. Among surviving patients (n = 43), the median follow-up time after diagnosis was 17.8 months (range 5.23−39.83 months). In our cohort, 44 patients (48.4%) died, including 3 (16.7%) of a total of 18 patients with p16 immunoreactivity and 37 (56.9%) of 65 patients without p16 immunoreactivity ($P = .002$) (see Table II). The Cox regression analysis revealed that HPV− patients had a 4.13-fold increased risk of death caused by OPSCC compared with HPV+ patients ($P = .01$) (Table III). The log-rank result results corroborated this and showed that the DSS survival curve of HPV+ patients was more favorable compared with that of HPV− cases ($P = .01$) (Figure 2A). Other factors significantly associated with DSS were presence of nodal metastasis on initial cervical findings, resectability/operability, size (T), nodal metastasis (N), and AJCC TNM (tumor−node−metastasis) stage (see Table III; Figure 2B and Figure 3). Kaplan-Meier cumulative DSS curves, according to the *AJCC Cancer Staging Manual* 8th edition, were well separated for both HPV+ cases (Figure 4A) and HPV− cases (Figure 4B), although the low specific mortality compromised statistical significance in the HPV-positive group. The AJCC system showed the highest hazard ratio, with patients with stages III and IV having a 22.18-fold increased chance of death caused by OPSCC compared with patients in stages I and II. In this context, AJCC staging status supplanted HPV status as the feature that presented the most significant association with survival in

Table II. Association of HPV status and demographic, clinic and pathologic features of patients with OPSCC

	HPV+	*HPV−*	*P value*
Gender, n (%)			
Male	14 (73.7%)	61 (89.7%)	.08
Female	5 (26.3%)	7 (10.3%)	
Age, in y			
Mean ± SD	60.8 ± 10.32	61.6 ± 8.14	.72
Range	49− 84	44− 81	
Skin color, n (%)			
Caucasian	19 (100%)	64 (94.1%)	.36
Non-Caucasian	0 (0%)	4 (5.9%)	
Educational level			
Primary school	15 (78.9%)	57 (83.8%)	.42
High school/Bachelor's degree	4 (21.1%)	11 (16.2%)	
Smoking status, n (%)			
Yes/former user	11 (57.9%)	66 (97.1%)	**< .001**
No	8 (42.1%)	2 (2.9%)	
Alcohol use, n (%)			
Yes/former user	7 (38.9%)	60 (88.2%)	**< .001**
No	11 (61.1%)	8 (11.8%)	
Histologic grade, n (%)			
Well-differentiated	4 (25.0%)	15 (23.1%)	**.01**
Moderately differentiated	7 (43.8%)	46 (70.8%)	
Poorly differentiated	5 (31.2%)	4 (6.2%)	
Initial symptoms, n (%)			
Upper aerodigestive tract	9 (47.4%)	49 (72%)	**.02**
Adenopathy	10 (52.6%)	15 (22.1%)	
No symptoms/casual diagnosis	0 (0%)	4 (5.9%)	
Site, n (%)			
Lateral pharyngeal walls	13 (68.4%)	28 (41.8%)	.19
Soft palate/uvula	2 (10.5%)	14 (20.9%)	
Posterior pharyngeal walls	0 (0%)	4 (6%)	
Base of the tongue/ vallecula	4 (21.1%)	21 (31.3%)	
Duration of symptoms, in months			
Mean ± SD	6.71 ± 8.33	5.89 ± 5.15	.34
Range	0.5−24	0.5−24	
Initial oral findings, n (%)			
Suspicious lesion	17 (89.5%)	66 (97.1%)	.20
Unsuspicious lesion	2 (10.5%)	2 (2.9%)	
Initial cervical findings, n (%)			
Suspicious lesion	14 (73.7%)	51 (75%)	.56
Unsuspicious lesion	5 (26.3%)	17 (25%)	
Resectability/operability, n (%)			
Unresectable/ inoperable	2 (10.5%)	29 (42.6%)	**.008**
Resectable/operable	17 (89.5%)	39 (57.4%)	
T, n (%)			
T1/T2	11 (57.9%)	22 (32.4%)	**.04**
T3/T4	0 (42.1%)	46 (67.6%)	
N, n (%)			
cN/pN 0	4 (21.1%)	15 (22.1%)	.59
cN/pN +	15 (78.9%)	53 (77.9%)	
TNM, n (%)			

(*continued*)

Table II. Continued

	HPV+	*HPV−*	*P value*
I/II	12 (63.2%)	10 (14.7%)	< **.001**
III/IV	7 (36.8%)	58 (85.3%)	
Treatment, n (%)			
Surgery (with or without RT/CT)	8 (44.4%)	17 (32.7%)	.26
Radiotherapy (with or without SX/CT)	10 (55.6%)	35 (67.3%)	
Metachronous or synchronous tumor, n (%)			
Present	5 (26.3%)	11 (16.2%)	.24
Absent	14 (73.7%)	57 (83.8%)	
Outcome, n (%)			
Alive	15 (83.3%)	28 (43.1%)	**.002**
Death caused by the tumor	3 (16.7%)	37 (56.9%)	

CT, Chemotherapy; *RT*, Radiotherapy; *SX*, Surgery; *HPV,* human papillomavirus; *OPSCC,* oropharyngeal squamous cell carcinoma; *SD,* standard deviation; *TNM,* tumor−node−metastasis.
Bold *P* values significant <0.05.

the multivariate analysis (Table IV). When AJCC and p16 stages were analyzed together, the p16 stage lost its significance with regard to its association with poor DSS, whereas the AJCC stage remained significant, with a hazard ratio of 19.95.

DISCUSSION

The prevalence of high-risk HPV DNA in OPC and OC varies according to geographic regions. HPV-related OPSCC prevalence has been reported to be highest (approximately 62%−77%) in North America, northern Europe, New Zealand, and Australia[10,12,24-29]; intermediate (approximately 37% to 50%) in Asia, Oceania, and Central Europe[17,30-32]; and low (approximately 15%) in South and Central America.[33-36] Brazilian data are scarce, and often in previous studies, the results are based mainly on small series, the methods employed for case identification vary, and it is difficult to differentiate studies that enrolled consecutive patients from studies that used alternative inclusion criteria. Furthermore, misclassification of advanced OPC as OC might have compromised the accurate determination of the prevalence rates of HPV infection in some series because the difference in HPV prevalence rates between HPV in the oropharynx and the other sites may be 4 times higher, especially in regions with high HPV prevalence.[20,37] We found only 3 Brazilian studies exploring the prevalence of HPV status in OPSCC with clearly consecutive series of cases. The largest Brazilian series was part of a large, multi-institutional study. In a multicentric Latin American cohort, 252 OPSCC cases were found among those selected from Goiania, Rio de Janeiro, São Paulo and Ribeirão

Table III. Prognostic value for OPSCC disease-specific survival (DSS)

	DSS Univariate analysis	
	HR (95% CI)	*P*
Gender		
Male	1	.71
Female	1.16 (0.51−2.60)	
Age, in years	1.02 (0.99−1.06)	.13
Educational level		
Primary school	1	.07
High school/Bachelor's degree	0.39 (0.14−1.10)	
Smoking status		
No	1	.42
Yes/former user	1.62 (0.50−5.25)	
Alcohol use		
No		
Yes/former user	1	.50
	1.29 (0.60−2.78)	
Histologic grade		
Well-differentiated	1	.08
Moderately differentiated	1.98 (0.90−4.37)	.50
Poorly differentiated	0.59 (0.12−2.79)	
Site, n (%)		
Lateral pharyngeal walls	1	.30
Soft palate/uvula	1.53 (0.67−3.47)	.18
Posterior pharyngeal walls	2.32 (0.67−7.99)	.09
Base of the tongue/vallecula	1.82 (0.89−3.68)	
Duration of symptoms, in months	0.97 (0.91−1.02)	.28
Initial oral findings		
Unsuspicious lesion	1	.60
Suspicious lesion	1.46 (0.35−6.05)	
Initial cervical finding		
Unsuspicious lesion	1	**.003**
Suspicious lesion	4.89 (1.74−13.75)	
Resectability		
Resectable	1	< **.001**
Unresectable	5.32 (2.08−10.01)	
T		
T1/T2	1	< **.001**
T3/T4	4.91 (12.06−11.67)	
N		
cN/pN 0	1	**.005**
cN/pN +	5.49 (1.68-17.84)	
TNM		
I/II	1	**.002**
III/IV	22.18 (3.02−162.40)	
Treatment		
Surgery (with or without RT/CT)	1	.08
Radiotherapy (with or without SX/CT)	2.27 (0.90−5.71)	
Other head and neck primary tumor		
Absent	1	.10
Present	0.46 (0.18−1.17)	
HPV status based on p16		
Positive	1	**.01**
Negative	4.13 (1.27−13.41)	

CT, chemotherapy; *HPV,* human papillomavirus; *OPSCC,* oropharyngeal squamous cell carcinoma; *RT*, Radiotherapy; *SD,* standard deviation; *SX*, Surgery; *TNM,* tumor−node−metastasis.
Bold *P* values significant <0.05.

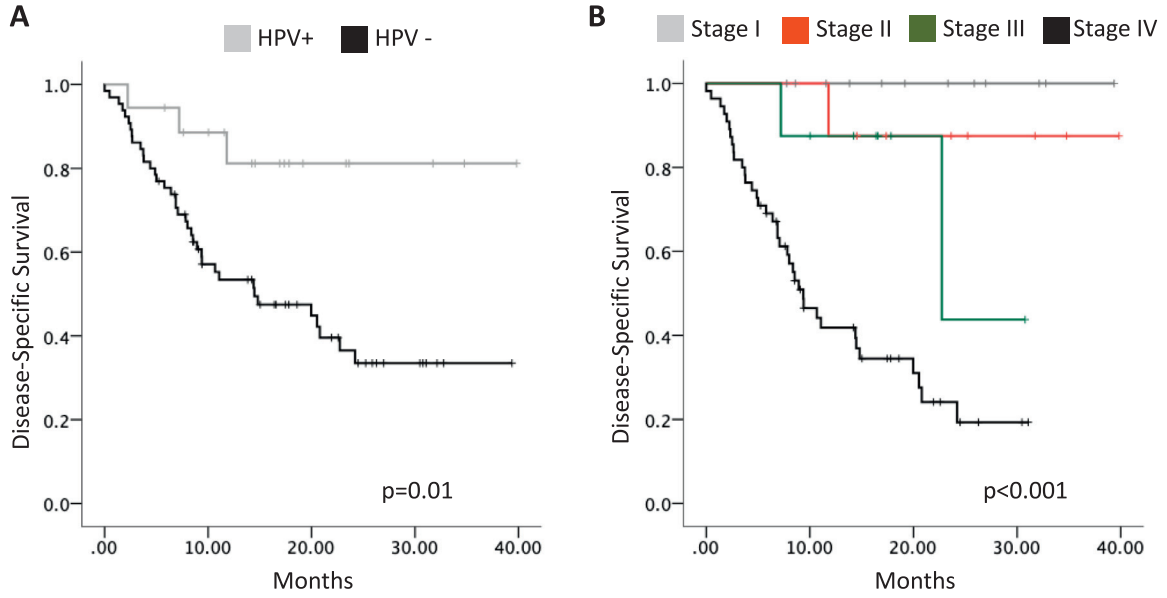

Fig. 2. Kaplan-Meier cumulative disease-specific survival curve according to human papillomavirus (HPV) status (A) and AJCC 8th edition staging system (B). *P* value based on Log-rank test.

Preto, from 1998 to 2008. These cases had an HPV-related HPV prevalence of 4.4% (4 of 91) when analyzing HPV-16 DNA and p16 by using IHC staining,[34] although in the majority of cases (n = 161), the authors had no tissue available for testing. Another multicentric study used data from the Brazilian Head and Neck Genome Project (GENCAPO, Sao Paulo, Brazil) study, which recruited patients with HNC and matched controls from 2002 to 2015. The study included 171 OPSCC cases, with a 4.1% prevalence of HPV identified with polymerase chain reaction (PCR) and p16-IHC.[32] The last study from Goiânia, Goiás, in which Petito et al.,[38] working only with PCR DNA extraction, found the highest prevalence of HPV-related OPSCC reported in Brazil (11 of 43; 25.5%). In our study, we found an overall prevalence of 20.9%, exclusively on p16 IHC staining. Our results are in accordance with those from previous Brazilian cohorts, apparently showing a gradual increase in HPV-related OPSCC prevalence over the last 2 decades. Interestingly, analyzing the annual prevalence, we observed an increase from 21.1% in 2017 to 32.4% in 2019, an increase of greater than 11% in a 3-year period.

In 2005, Kreimer et al.[37] published a systematic review exploring the geographic heterogeneity in HPV-status in OPSCC. Those authors suggest that the differences in HPV-prevalence among patients with OPSCC might be partly explained by regional differences in the distribution of risk factors other than HPV infection. The United States is currently the only country with significant studies reporting time-based trends in oral sexual behavior. Studies from the 1940s to the present day appear to support the notion of an increase in oral sexual behavior. The number of men who ever engaged in oral sex rose from 10% in the 1940s/1950s to approximately 50% by the 1970s/1980s and continued to rise to 75% by 1991 and 85% by 2010. These changing sexual practices may explain the observed trends in the prevalence of HPV+ OPSCCs in North America and Europe. In comparison, these practices may be changing more gradually across other nations around the world.[8] Rather than offering the simplistic explanation of changing sexual practices, Syrjänen et al. proposed an alternative concept, which proposes that the timing of the first HPV infection and HPV-specific immunity may play a key role in the pathogenesis of OPSCC. According to those authors, World Wars I and II increased the burden of HPV infection of the genital and oral tracts as a result of the crowding of the people both at home and in the war zones. The children of the postwar "baby boomers" (those born between 1945 and 1950) were at increased risk for vertical HPV transmission when the returning soldiers infected their wives or partners with HPV shortly before their first pregnancy. One can estimate that the burden of HPV infection increased substantially during these decades, leading to an increase in HPV-associated carcinomas some 30 to 50 years later. If we anticipate HPV being also vertically acquired during that period, the increase in the incidence rate of OPSCC should have started in the late 1960s (in the post−World War I birth cohorts) and in the 1990s (in the post−World War II birth cohorts), exactly as has been shown to have happened in many countries.[39] If this theory is correct, because the incidence of cervical cancer remains high in Brazil[4] and the adherence to HPV vaccination is still low,[40] it is predicted that the incidence of HPV-related OPSCC will continue to increase in the

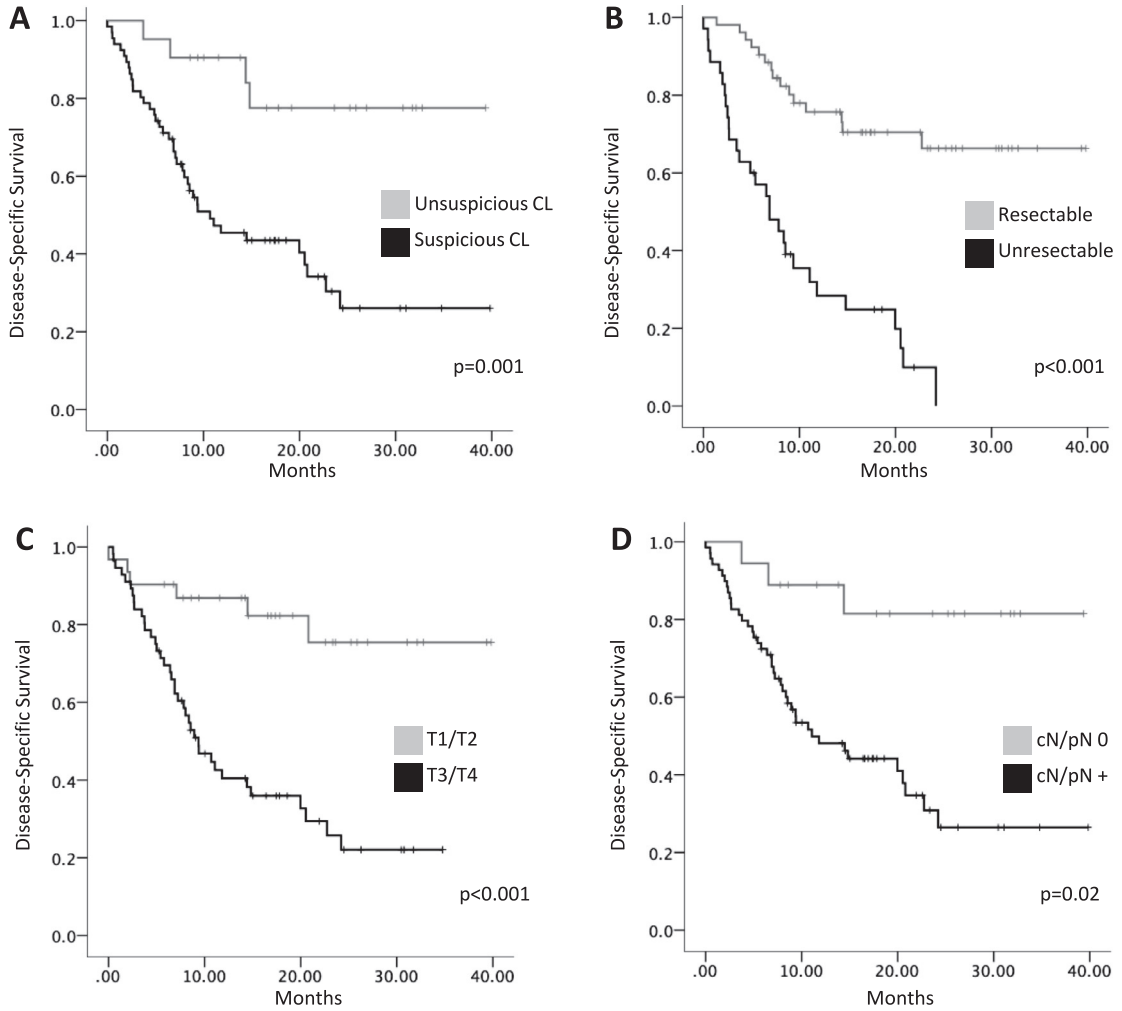

Fig. 3. Kaplan-Meier cumulative disease-specific survival curve according to AJCC 8th edition staging system in HPV-positive cases (A) and HPV-negative cases (B). *P* value based on Log-rank test.

coming years in Brazil. In addition, it is expected that with the reduction in the incidence of cervical cancer in developed countries,[41-43] stabilization or reduction in the incidence of HPV-associated OPSCC could be possible, a fact that, so far, has been observed in one isolated study from Stockholm,[44] one of the world's regions with the highest incidence.

Various methodologic approaches are currently used to identify HPV-induced HNC. The 8th edition of the *AJCC Cancer Staging Manual* introduced p16-based categorization of OPC into cases associated with high-risk HPV and those that are not. The surrogate marker p16 was chosen because of the lower cost, widespread availability, and relative ease of interpretation, as opposed to specific determination of high-risk HPV.[45] Although consistent discrepancies in p16 positivity and HPV positivity assessed through more robust methods, such as DNA in situ hybridization, have been observed in HNC,[46] there is robust evidence indicating that even HPV−/p16+ head and neck squamous cell carcinoma (HNSCC) cases have better survival outcomes compared with HPV+/p16− and HPV−/p16− cases.[47] Furthermore, a meta-analysis aiming to estimate the diagnostic accuracy of p16 INK4a IHC to detect transformation of HPV infection to carcinomas of oropharyngeal origin showed no statistically significant heterogeneity between the groups with respect to sensitivity or specificity of p16 INK4a IHC compared with the gold standard HPV oncogene mRNA detection.[46]

For unclear reasons, p16 has been demonstrated to be an independent surrogate marker. Although p16 IHC analysis has been replacing more intensive HPV DNA in situ hybridization and PCR-based methods for the assessment of HPV status, the method is prone to false-positive results. In the latter context, encountering elevated p16 expression caused by nonviral−related alterations must be considered. According to El-Naggar et al., absent or weak p16 staining in the OPC of basaloid nonkeratinized/partially keratinized

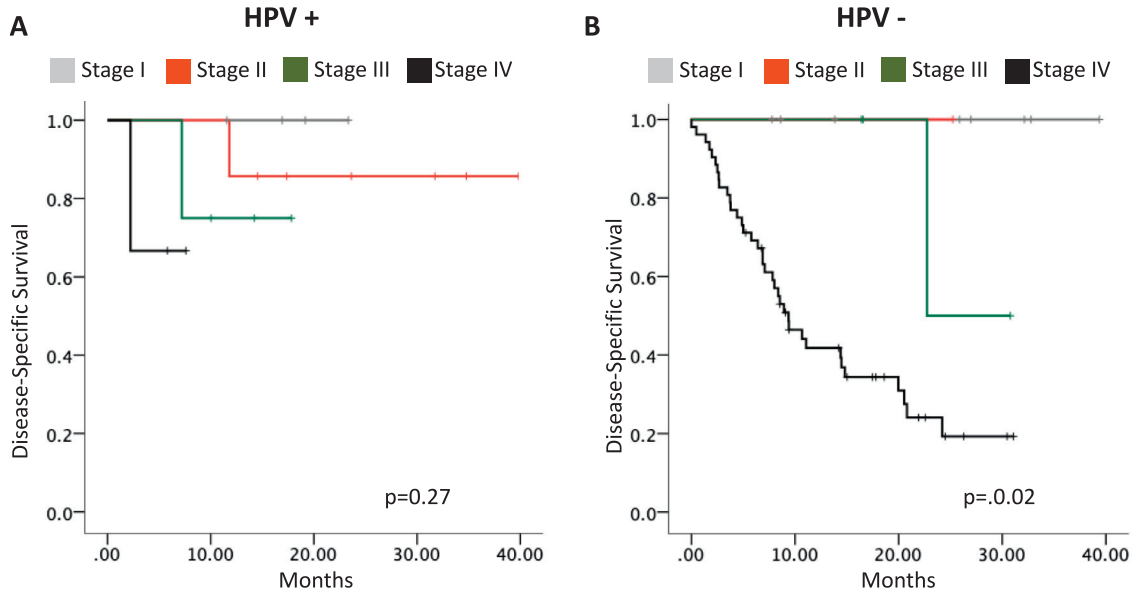

Fig. 4. Kaplan-Meier cumulative disease-specific survival curves according to presence of cervical lesion (CL) (A), tumor resectability (B), size (T) (C), nodal metastasis (N) (D). *P* values based on Log-rank test.

Table IV. Multivariate model of HPV and AJCC stage prognostic value for OPSCC disease-specific survival (DSS)

	DSS Multivariate analysis	
	HR (95% CI)	*P*
TNM		
I/II	1	**.002**
III/IV	19.05 (2.45−147.73)	
HPV status based on p16		
Positive	1	.56
Negative	1.42 (0.24−4.80)	

AJCC, American Joint Committee on Cancer; *CI,* confidence interval; *HPV,* human papillomavirus; *HR,* hazard ratio; *OPSCC,* oropharyngeal squamous cell carcinoma; *TNM,* tumor−node−metastasis.
Bold *P* values significant <0.05.

phenotypes requires additional HPV testing and p16 staining in conventional keratinizing SCC of the oropharynx.[48]

The longstanding concept that HNC presents as a homogeneous tumor entity is changing. Over the last few decades, accumulating evidence has established HPV as a major etiologic factor in a subset of HNSCCs, in particular, those that arise from the oropharynx.[49,50] The high-risk genotype HPV-16 accounts for the vast majority (approximately 90%−95%) of HPV+ OPSCC cases.[50] Particularly in developed nations, a recent dramatic rise in the incidence of HPV-related OPSCC has caused concerns about an emerging cancer epidemic. In the United States, the prevalence of HPV-related OPSCC increased from 16% of cases in the early 1980s to greater than 60%, as shown by more recent studies.[50] An increase in the incidence of oropharyngeal cancer has also been observed in some European countries over the past 2 decades. As indicated by the study of Xu et al., the burden of OPC in the U.S. population is predicted to increase sharply over the next 30 years. Among men age 70 years and older a great increase of OPC diagnoses is projected, with 354% more OPC cases expected to be diagnosed from 2016 to 2045. By 2045, OPC is projected to be the third most common cancer in non-Hispanic white men age 55to 69 years.[51] Data from southern Brazil concerning the prevalence of HPV+ cases among patients with OPSCC and data concerning the behavior of these tumors are limited. Our study showed a slight increase in the rate of HPV+ cases seen at our institution during a 3-year period and also the better prognosis for patients with HPV+ OPSCC compared with HPV− OPSCC cases.

Patients with HPV-associated tumors show different tumor biology and risk factor profiles compared with patients with HPV− tumors.[52,53] Most studies have shown a male predilection in both groups,[24,54,55] although the proportion of females was higher in the HPV+ group in our study (P = .08), similar to the results of Lam et al.[56] This difference may be partially explained by the presence of tobacco and alcohol exposure (76.9% and 38.4% in women vs 91% and 83.3% in men, respectively) in our sample. HPV+ tumors are more likely to occur in fair-skinned patients,[54-56] younger individuals,[10,12,24,25,29,44,54-56] those of higher socioeconomic status,[54,55] and those with minimal or no history of smoking and drinking.[12,17,24,25,28,29,32,57,58] Our results confirmed a higher prevalence of smoking (P < .001) and alcohol (P < .001) consumption in the HPV− group. HPV−

cases had a higher mean age, lower educational level, and lower percentage of fair-skinned individuals, although there was no statistical significance (P = .72, .42, and .36, respectively).

HPV+ tumors are more likely to present at earlier T stages and advanced N stages.[10,12,17,29,44,54-57] As reported in other studies,[28,59,60] patients with HPV+ OPSCC were more likely to have a neck mass as the chief complaint, whereas HPV− patients were more likely to have sore throat as the chief complaint at the first clinical presentation (P = .02). In our experience, HPV+ cases were more likely to present in earlier T (P = .04) and clinicopathologic (P < .001) stages. Advanced disease is common in our study region, with 38.5% of the cases presenting with unresectable/inoperable tumors and 71.4% in stages III and IV. Although we did not find any studies comparing resectability rates in this HPV-associated oropharyngeal SCC scenario, in the HPV− group, the prevalence of unresectability/inoperability was significantly higher (42.6% vs 10.5%; P = .008). Investigating predictors of HNSCC survival in Brazil, Argentina, Uruguay, and Colombia, the InterCHANGE study showed 87.5% prevalence of stages III and IV in OPSCC.[61] Reasons for the late diagnosis of HNSCC in South America are often multifactorial and may include lack of awareness of cancer signs and symptoms (in both patients and health care providers), lack of access to appropriate health care, and shortage of medical resources.[61] Analyzing survival trends of patients with OC and OPC treated at a cancer center in São Paulo, Brazil, Kowalski et al. found similar results, with 90% (339 of 380) of patients with OPSCC in stages III and IV.[62]

In our experience, regional disease is highly prevalent (78%), and no differences in N stage were observed between the 2 groups in our sample (P = .59). Although tumor grading does not provide any correlation with clinical behavior in HPV scenario,[63] in our cohort, 31.2% of HPV+ OPSCC cases were graded as poorly differentiated compared with only 6.2% in the HPV− group (P = .01), corroborating the literature concerning a higher histologic grade in this type of tumor.[24,25,28,32,54,55,57]

The lateral pharyngeal wall was the predominant site in both groups, with a higher prevalence in HPV+ cases (68.4% vs 41.8%), corroborating the findings reported in the literature,[12,17,25,28,29,32,44,54,55,57] although without statistical significance (P = .19). A poorly explored issue is the comparison of the rates of metachronous or synchronous SCCs, according to HPV status in OPSCC. Lam et al.[56] observed that synchronous and metachronous tumors were more commonly found in the non-HPV group, although the differences did not reach statistical significance. Our results indicated the opposite: Although also without statistical significance, metachrony and synchrony were more common in the HPV+ group (26.3% vs 16.2%; P = .24).

Studies from regions around the world have shown better outcomes in patients with HPV+ OPC compared with those with corresponding HPV− tumors, with rates around 80% to 90% vs 40% to 50% for a 5-year OS rate.[12,17,29,32,57-58,64-66] Despite our 17.8-month median follow-up, HPV− status was associated with worse outcomes in the survival analysis. The 2-year DSS rate was 81% in the HPV+ group compared with 67% in the HPV− group.

The main limitations of our study are related to the follow-up period, sample size, and absence of p16 status in 4 patients in our sample. A longer follow-up period would be important to determine if this difference in survival rate is stable over time. However, it is also interesting to note that in just a short period after diagnosis, it was possible to see significant differences in the chances of survival according to HPV status. As expected, higher T stage (P < .001), higher N stage (P = .005), higher AJCC stage (P = .002), and higher unresectable/inoperable disease stage (P < .001) were all associated with worse survival rates. In this context of a low prevalence of HPV-associated OPSCC, AJCC staging status supplanted HPV status as the feature that presented the most significant association with survival in the multivariate analysis (see Table IV).

CONCLUSIONS

The prevalence of HPV+ OPSCC, based on the results of p16 IHC, in southern Brazil is relatively low compared with that in the United States and Europe. However, it is similar to the levels reported in other Brazilian centers and seems to show a trend toward a gradual increase in recent years. The current analysis showed a favorable prognosis for patients with p16-positive OPSCC compared with p16-negative cases, although this was not independent from the AJCC staging status, which achieved the highest hazard ratio for death caused by OPSCC.

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