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Nodal involvement and p16-staining in upper alveolar ridge and hard palate cancer

Edgar Salas¹, Pedro Sanchez¹, Juan Postigo¹, Carlos A. Castaneda^{2,3}, Miluska Castillo², Valeria Villegas², Luis Cano², Sandro Casavilca⁴, Luis A. Bernabe², Carolina Belmar², Maria R. Villa-Robles⁴, Raul Mantilla², Henry Guerra⁴

¹Head & Neck Surgery Department, Instituto Nacional de Enfermedades Neoplasicas, Lima 15038, Peru.

²Research Department, Instituto Nacional de Enfermedades Neoplasicas, Lima 15038, Peru.

³Faculty of Medicine, Universidad Peruana San Juan Bautista, Lima 15067, Peru.

⁴Pathology Department, Instituto Nacional de Enfermedades Neoplasicas, Lima 15038, Peru.

Correspondence to: Dr. Carlos A. Castaneda, Medical Oncology Department and Research Department, Instituto Nacional de Enfermedades Neoplasicas, Av. Angamos Este 2520 Surquillo, Lima 15038, Peru. E-mail: carloscastanedaaltamirano@yahoo.com

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Abstract

Aim: Upper alveolar ridge and hard palate squamous cancer is an infrequent malignancy. We evaluated factors associated with neck involvement and with p16-staining.

Methods: Head and neck squamous-cell carcinoma (SCC) patients who went to Head and Neck Department between 1997 and 2011 were screened, and 73 resected upper alveolar ridge and 5 hard palate SCC were selected. Tumors with available tissue were stained with p16 immunohistochemistry.

Results: Median age was 64.4 years, 55.1% were female, and 73.1% were in clinical stage IV. Neck dissections were performed in 24 and pathologically confirmed node metastases were found in 19 (24.3%). Cervical recurrence was found in 18 patients (23.1%) and was associated with histological grade ($P = 0.037$). Three (7.3%) of 41 lesions were positive for p16 and tended to be younger ($P = 0.067$). Lymphovascular invasion was associated with shorter disease-free survival (DFS) ($P = 0.026$) and overall survival (OS) ($P = 0.021$). Larger cT ($P = 0.019$), perineural invasion ($P = 0.039$) and neck dissection ($P = 0.010$) were associated with shorter OS. Neck node involvement tended to have shorter DFS (31% vs. 48.7%, $P = 0.278$) and OS (25.1% vs. 48.5%, $P = 0.340$), and neck recurrence tended to have shorter OS (9.3% vs. 52.3%, $P = 0.064$).

Conclusion: Neck involvement and recurrence are frequent in this location. P16-positive cases were present in 7.3% and tended to be associated with younger age.



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Keywords: P16, head, neck, maxilla, prognosis, survival

INTRODUCTION

Squamous cell carcinomas (SCC) of the hard palate and upper alveolus ridge are relatively rare^[1]. Prognostic factors and neck management in head and neck SCC (HNSCC) have been extensively studied in series of tongue or floor of mouth SCC, or on series with a mixture of SCC tumor sites^[2]. Only small retrospective series have evaluated the behavior of hard palate and upper alveolus, and suggest that they have a low rate of regional node metastases^[3-8]. However, recent studies find higher rates of both neck lymph node involvement and neck recurrence in these malignancies, and, there is a need to identify those aggressive cases that would benefit from more aggressive treatment^[9,10].

Several clinicopathological features have been implicated in recurrence risk and prognosis in HNSCC. These include tumor size, nodal involvement, tobacco and alcohol consumption, and presence of human papillomavirus (HPV) infection^[11,12].

The prevalence of HPV infection is higher in oropharyngeal squamous cell carcinoma (OPSCC) (35.6%) and has been associated with both better prognosis and higher response rate to chemoradiation^[12,13]. P16 staining is highly correlated with HPV infection in OPSCC and has also been associated with good prognosis^[14-17]. There is no information about the rate of p16 expression in rare locations like hard palate and upper alveolar ridge.

The aim of the present study was to evaluate predictive factors associated with node involvement, prognostic factors, and prevalence of p16 staining in hard palate and upper alveolar ridge SCC.

METHODS

Study population

All patients treated at Department of Head and Neck at Instituto Nacional de Enfermedades Neoplásicas with maxillary SCC between January 1997 and December 2011 were screened for the study. Inclusion criteria included having a primary tumor located in the upper alveolar ridge or hard palate, having a squamous histology, and having history of resection of primary tumor. Patients with primary tumor of nasal cavity and paranasal sinuses were excluded. The procedure of neck management was selected by the Institute surgeon. It included neck dissection in cases of clinically involved lymph nodes and in cases of metastasis risk factors like greater depth of primary tumor deep invasion. Selection of ipsilateral or bilateral dissection was also determined by the Institute surgeon and took into account clinical factors like proximity to midline.

Information about clinicopathological variables was taken from patient files and pathology report. Data included age, gender, tobacco use, alcohol use, tumor subsite, depth of invasion, histologic grade, margin status, perineural invasion (PNI), lymphovascular invasion (LVI), clinical and pathological stage (TNM classification), surgical procedure, radiation or chemotherapy administration, and date of last follow-up or death. Some standard pathological features that were not reported in patient file were prospectively completed by a pathologist (LC). The institutional review board approval was obtained from The Instituto Nacional de Enfermedades Neoplásicas (Lima, Peru). Since the study was based on a secondary source and there was no contact with the patients, no informed consent was applied; however, the identity and personal data of patients' medical records were protected at all times.

P16 immunohistochemistry assay

Pathologists evaluated H&E slides under light microscopy and the most representative tissue were selected. A 0.6-cm punch was taken from each formalin-fixed paraffin-embedded (FFPE) sample selected and was

transferred to an empty paraffin recipient block in order to construct tissue microarrays (TMA). FFPE samples were fixed for 6 to 8 h in 10% neutral buffered formalin and routinely processed with standard methodologies.

In total, 41 tissue cores were distributed into ten TMA blocks. Tissue sections were cut at 3 mm and float-mounted on adhesive (silanized) glass slides. Immunohistochemistry (IHC) for p16 status was performed using the DAKO EnVision™ FLEX+ detection system together with the Autostainer Link instrument (DAKO Corp, Carpinteria, California) on FFPE tissue. Antigen was retrieved using EnVision™ FLEX Target Retrieval Solution, High pH, and p16 was detected using p16 mouse monoclonal antibody (clone 16p04, JC2, BSB 5828, prediluted, Bio SB, Santa Barbara, California). The EnVision™ FLEX+, Mouse, High pH, (LINK) Kit was used to perform the assay according to the manufacturer's instructions. It contains the substrate chromogen 3-3'-diaminobenzidine (DAB), which, on staining, results in a brown-colored precipitate at the antigen site.

Positive p16 expression was defined as a strong and diffuse nuclear and cytoplasmic staining in at least 70% of the tumor cells.

Immunohistochemical evaluation was carried out by three pathologists in independent readings (LC, HG, and SC). Reports that varied among readers were reevaluated to determine a consensus.

Statistical analysis

The log-rank statistic was used for univariate analysis, and Cox proportional hazards regression was used for multivariable analysis. Categorical comparisons were carried out using the chi-square statistic or Fisher exact test. In all cases, the level of alpha was set at 0.05 a priori. Survival analysis was calculated using the Kaplan-Meier method. All analyses were performed in SPSS version 17.0 (SPSS, Chicago, IL).

RESULTS

Tumor primary location for this cohort was distributed in 5 patients for hard palate and 73 for upper alveolar ridge. Mean age was 64.47 years old and 55.1% were female. There were tobacco and alcoholism history in 10.3% and 6.4% cases, respectively. Two (40%) hard palate and 52 (71.2%) upper alveolar ridge tumors were clinically classified cT4, and 3 (60%) hard palate and 21 (28.8%) upper alveolar ridge tumors were clinically node-positive at presentation. Clinical stages I-IV of upper alveolar ridge SCC were found in 1 (1.4%), 13 (17.8%), 5 (6.8%) and 54 (74%) of cases, respectively. Clinical stages II-IV of hard palate SCC were found in 1, 1 and 3 cases, respectively [Table 1].

The primary tumor was resected in all cases ($n = 78$). Neck dissections were initially performed in 24 cases (21 in clinically node-positive and 3 in node-negative). Nineteen (79.16%) of cases who went to neck dissections had confirmed nodal metastases on pathological examination (including the 3 clinically node-negative cases). Cervical metastases in these 19 node-positive cases were distributed between levels I (94.7%), II (73.7%), and III (26.3%). Extracapsular extension at presentation was noted in 7 specimens of upper alveolar ridge tumors.

In no instances were age ($P = 0.329$), location ($P = 0.590$), cT ($P = 0.629$), histological grade ($P = 0.361$), PNI ($P = 0.825$), or LVI ($P = 0.080$) associated with cervical metastases [Table 2].

Neck recurrences

Altogether, 18 patients (75%) developed cervical recurrences, and 8 (44.4%) of them went to neck dissection rescue (3 of them with additional radiation). Altogether, 18 patients (75%) developed cervical recurrences and 8 (44.4%) of them went to neck dissection rescue (3 of them with additional radiation). Ten patients did not go to surgery and treatment for them were: radiation alone ($n = 2$), radiation and chemotherapy

Table 1. General features

	Cases	%
Age (years), mean (range)	64.47 (21-89)	
Gender		
Female	43	55.1
Male	35	44.9
Tobacco		
Yes	8	10.3
No	70	89.7
Alcohol		
Yes	5	6.4
No	73	93.6
Location		
Hard palate	5	6.4
Upper alveolus	73	93.6
cT		
T2-T3	22	28.2
T4	54	69.2
cN		
cN0	54	69.2
cN-positive	24	30.8
Clinical stage		
I	1	1.3
II	14	17.9
III	6	7.7
IV	57	73.1
Histological grade		
Poor/moderate differentiation	34	43.6
Well differentiated	44	56.4
Perineural infiltration		
No	31	39.7
Yes	21	26.9
Lymphovascular invasion		
No	40	51.3
Yes	13	16.7
p16		
Negative	38	48.7
Positive	3	3.8
Neck dissection		
No	54	69.2
Yes	24	30.8
Time from initial surgery to neck recurrence (months), mean (range)	8.6 (2-29)	
pN		
N0	9	11.5
N1	3	3.8
N2	15	19.2
N3	1	1.3
NX	50	64.1
Lymph node levels		
I	9	11.5
II	4	5.1
I, II	5	6.4
I, III	1	1.3
II, III	1	1.3
I, II, III	1	1.3
II, V, contralateral	1	1.3
I, II, III, contralateral	1	1.3
I, II, III, IV, contralateral	1	1.3
Without registration data	54	69.2
Extracapsular extension	7	29.2
N+ and N- with neck recurrence	33	42.3

Table 2. Relationship between clinicopathological features and lymph node involvement

	Neck lymph node (%)		<i>P</i>
	Negative (<i>n</i> = 59)	Positive (<i>n</i> = 19)	
Age (years), mean (range)	65.41 (31-89)	61.58 (21-88)	0.329
Location			
Upper alveolus	56 (94.9)	17 (89.5)	0.590
Hard palate	3 (5.1)	2 (10.5)	
cT			
T2-T3	19 (32.2)	5 (26.3)	0.629
T4	40 (67.8)	14 (73.7)	
Grade			
Poor/moderate differentiation	24 (40.7)	10 (52.6)	0.361
Well differentiated	35 (59.3)	9 (47.4)	
PNI			
No	23 (60.5)	8 (57.1)	0.825
Yes	15 (39.5)	6 (42.9)	
LVI			
No	32 (82.1)	8 (57.1)	0.080
Yes	7 (17.9)	6 (42.9)	
Tobacco			
No	53 (89.8)	17 (89.5)	1.000
Yes	6 (10.2)	2 (10.5)	
Alcohol			
No	55 (93.2)	18 (94.7)	1.000
Yes	4 (6.8)	1 (5.3)	
p16			
Negative	30 (90.9)	8 (100.0)	-
Positive	3 (9.1)	0 (0.0)	

PNI: perineural invasion; LVI: lymphovascular infiltration

(*n* = 2), chemotherapy alone (*n* = 2) or no-treatment (*n* = 4). Cervical metastases in these 18 cases of cervical recurrences were distributed between levels I (61.1%), II (100%) and III (27.8%).

Fourteen of the patients who were clinically node-negative (25.9%) and 4 of the patients who were pathologically confirmed node-positive at presentation (16.7%) had recurrences in the neck. The mean time to neck recurrence was 8.6 months (2 to 29 months). A factor associated with neck failure was high histological grade (*P* = 0.037). Recurrences were not associated with age (*P* = 0.725), cT (*P* = 0.754), N (*P* = 0.536), or PNI (*P* = 0.624) [Table 3].

Expression of p16

A total of 41 (52.5%) lesions were tested for p16 expression. Overall, 7.3% (3 of 41) were p16 positive: 1 of 2 in hard palate (50%) and 2 of 39 in alveolar ridge (5.1%) [Figure 1]. The p16 positive tumors were not associated with age (*P* = 0.067), tumor location (*P* = 0.143), cT (*P* = 1.000), or histological grade (*P* = 0.560) [Table 4].

Survival analysis

Median overall survival (OS) was 40 months. Neither smoking nor alcohol consumption was associated with shorter disease-free survival (DFS) (*P* = 0.815 and 0.507) nor OS (*P* = 0.597 and 0.634). LVI (*P* = 0.026) was associated with shorter DFS in univariate analysis. Larger cT (*P* = 0.019), presence of PNI (*P* = 0.039), LVI (*P* = 0.021), and neck dissection (*P* = 0.010) were associated with shorter OS in univariate analysis [Figure 2]. Neck involvement had a trend both for shorter DFS (31% vs. 48.7%, *P* = 0.278) and shorter OS (25.1% vs. 48.5%, *P* = 0.340). There was also a trend to shorter OS (9.3% vs. 52.3%, *P* = 0.064) in the presence of neck recurrence [Table 5].

Table 3. Relationship between clinicopathological features and neck recurrence (n = 78)

	Neck recurrence (%)		P
	No (n = 60)	Yes (n = 18)	
Age (years), mean (range)	64.8 (21-89)	63.4 (31-80)	0.725
cT			
T1-T2-T3	19 (31.7)	5 (27.8)	0.754
T4	41 (68.3)	13 (72.2)	
N			
N(-)	44 (73.3)	15 (83.3)	0.536
N(+)	16 (26.7)	3 (16.7)	
Location			
Upper alveolus	55 (91.7)	18 (100.0)	-
Hard palate	5 (8.3)	0 (0.0)	
Grade			
Poor/moderate differentiation	30 (50.0)	4 (22.2)	0.037
Well differentiated	30 (50.0)	14 (77.8)	
PNI			
No	24 (61.5)	7 (53.8)	0.624
Yes	15 (38.5)	6 (46.2)	
LVI			
No	30 (75.0)	10 (76.9)	1.000
Yes	10 (25.0)	3 (23.1)	
Tobacco			
No	52 (86.7)	18 (100.0)	-
Yes	8 (13.3)	0 (0.0)	
Alcohol			
No	56 (93.3)	17 (94.4)	1.000
Yes	4 (6.7)	1 (5.6)	
P16			
Negative	25 (89.3)	13 (100.0)	-
Positive	3 (10.7)	0 (0.0)	

PNI: perineural invasion; LVI: lymphovascular infiltration

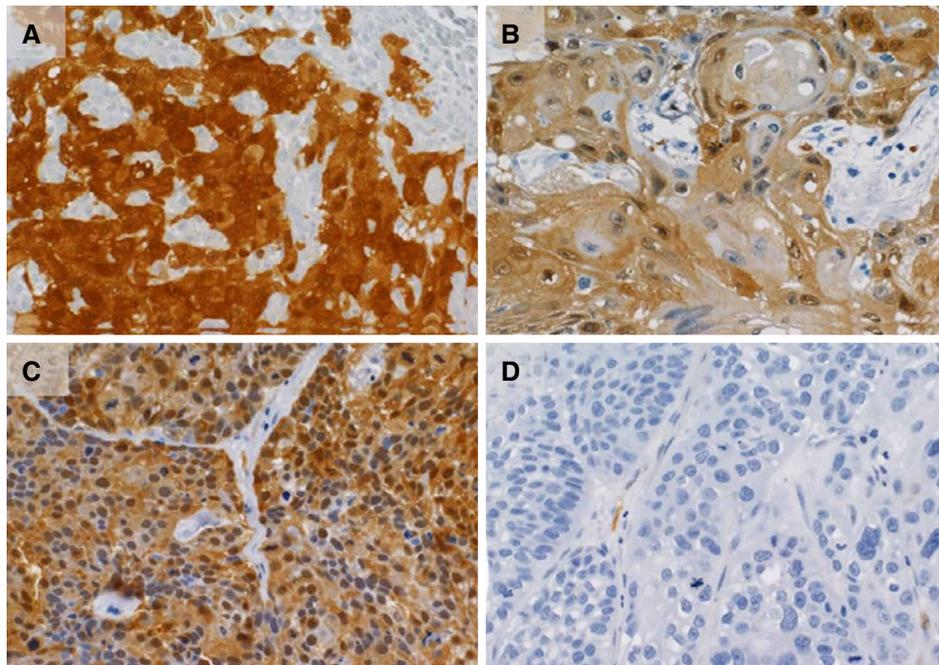


Figure 1. P16 by immunohistochemistry in upper maxilla. (A, B, C) Positive status of p16 staining indicated by brown staining of nuclear and cytoplasmic membrane in three cases; (D) negative status for p16 staining. (Magnification 40x)

Table 4. Relationship between p16 staining and clinicopathological features (n = 41)

	p16 staining (%)		P
	Negative (n = 38)	Positive (n = 3)	
Age (years), mean (range)	63.71 (21-89)	48.67 (44-55)	0.067
cT			
T1-3	10 (26.3)	1 (33.3)	1.000
T4	28 (73.7)	2 (66.7)	
cN			
N (-)	30 (78.9)	3 (100.0)	-
N (+)	8 (21.1)	0 (0.0)	
Location			
Upper alveolus	37 (97.4)	2 (66.7)	0.143
Hard palate	1 (2.6)	1 (33.3)	
Grade			
Poor/moderate differentiation	15 (39.5)	2 (66.7)	0.560
Well differentiated	23 (60.5)	1 (33.3)	
PNI			
No	12 (40.0)	1 (50.0)	1.000
Yes	18 (60.0)	1 (50.0)	
LVI			
No	22 (73.3)	2 (100.0)	-
Yes	8 (26.7)	0 (0.0)	
Tobacco			
No	35 (92.1)	2 (66.7)	0.271
Yes	3 (7.9)	1 (33.3)	
Alcohol			
No	35 (92.1)	3 (100.0)	-
Yes	3 (7.9)	0 (0.0)	

PNI: perineural invasion; LVI: lymphovascular infiltration

DISCUSSION

Our rate of survival at 5 years was 44.5% and is similar to those reported by other studies (21% to 76%)^[18,19]. This poor prognosis could reflect higher prevalence of neck node involvement at diagnosis or higher prevalence of poor prognostic factors like p16-negative status^[1].

The incidence of neck metastasis has been extensively described in cancer of the tongue and floor of the mouth (20% to 30%) and has been assigned a significant prognostic role in patients with clinically node-negative disease^[20,21]. Clinicopathologic factors like large tumor size, tumor depth, higher grade, and microvascular invasion have been associated with the development of cervical lymph node metastasis in oral SCC^[21]. Elective treatment of the neck with staging neck dissection is generally carried out in patients with SCC of the oral cavity when the risk of clinically occult metastases exceeds 15% to 20%, and treatment of the clinically N0 neck is now accepted for certain oral cavity subsites, such as the tongue and floor of mouth, where elective neck dissection produces a survival advantage^[22-26].

The understanding of the behavior of hard palate and upper alveolar cancers is poor due to their low incidence and because some of these studies indistinctly included different both other head and neck malignancy locations and special pathological entities like salivary gland tumors^[27,28]. A series of 606 upper and lower alveolar SCCs reported 37% of cervical metastasis and 19% of harbored occult disease in elective neck dissections. Lymph node involvement at level II to V carried shorter survival than negative lymph node involvement^[19]. A series of 347 cancers of the upper and lower gums that had an elective neck dissection rate of 58% found occult disease in 5.6%. Neck recurrence was found in 9% of the whole group. Ipsilateral and contralateral neck node involvement predicted cervical recurrence. Positive neck lymph nodes, tumor stage, and involved soft-tissue margins were significant covariates in survival prediction; clinical stage remained significant in multivariate analysis^[29]. A series of 252 cases of palate SCC including 62 in the specific region

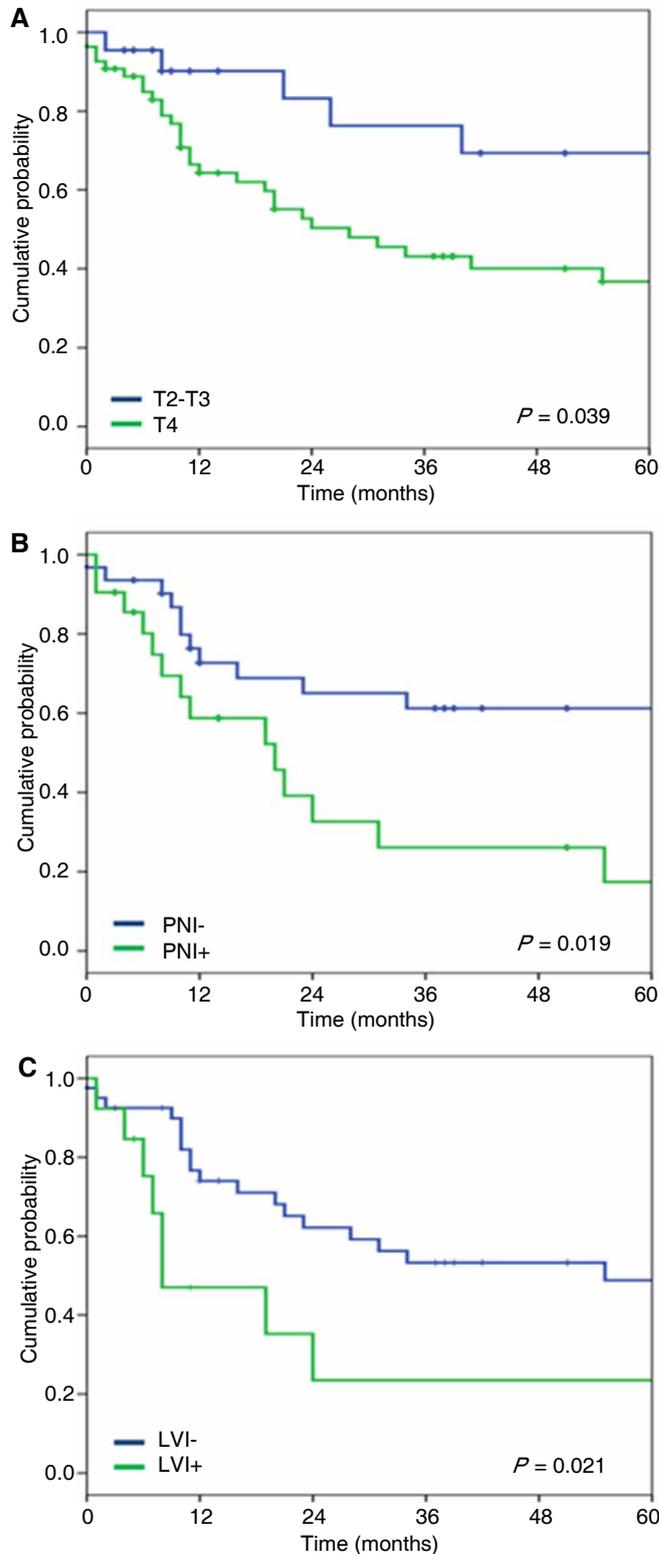


Figure 2. Estimated curves of OS regarding clinical tumor (A), perineural infiltration (B) and lymphovascular infiltration (C). OS: overall survival; PNI: perineural invasion; LVI: lymphovascular infiltration

of the hard palate found node involvement in more than 29% of the hard palate tumors. Neck recurrence was predicted by the presence of fixed or contralateral node metastases, but not by the presence of nodal metastasis itself. Size of the primary tumor and histological grade was significantly associated with survival,

Table 5. Survival analysis

	5-year-OS	P	5-year-DFS	P
All group	43.9%		44.5%	
Age				
≤ 60 years	56.1%		41.8%	
> 60 years	38.9%	0.667	45.8%	0.643
Gender				
Female	53.4%		51.0%	
Male	31.2%	0.539	37.8%	0.323
Tobacco				
Yes	21.9%		52.5	
No	46.2%	0.597	43.7	0.815
Alcohol				
Yes	0.0%*		40.0%**	
No	46.6%	0.634	44.8%	0.507
Location				
Hard palate	66.7%		53.3%	
Upper alveolus	42.7%	0.707	43.6%	0.851
cT				
T2-T3	69.3%		44.2%	
T4	36.7%	0.019	44.0%	0.743
Grade				
Poor/moderate differentiation	47.0%		51.7%	
Well differentiated	42.1%	0.715	39.9%	0.289
PNI				
No	61.2%		52.4%	
Yes	26.1%	0.039	36.3%	0.334
LVI				
No	48.8%		50.9%	
Yes	23.5%	0.021	24.7%	0.026
P16				
Negative	27.9%		35.0%	
Positive	100.0%	-	100.0%	-
Neck dissection				
No	53.4%		50.1%	
Yes	18.8%	0.010	29.6%	0.129
pN				
N-	48.5%		48.7%	
N+	25.1%	0.340	31.0%	0.278
Neck recurrence				
No	52.3%			
Yes	9.3%	0.064		
N+ and N- with neck recurrence				
No	55.5%			
Yes	21.6%	0.192		

*Estimated at 41 months; **estimated at 9 months. PNI: perineural invasion; LVI: lymphovascular infiltration

and clinical stage was the most important prognostic indicator^[30]. Recent retrospective series with 26 to 146 upper alveolar ridge and hard palate cases reported a neck lymph node involvement between 11% and 36.6%, and regional recurrence in N0 neck from 14% to 27%^[1,5-8]. These studies had several findings: cases with neck node involvement had higher grade; clinical stage but not margin status was associated with prognosis; and T3 (55%) and T4 (52%) tumors exhibited higher rates of neck lymph node involvement than smaller tumors (T1 = 15%; T2 = 28%). An analysis of the Surveillance, Epidemiology, and End Results (SEER) database evaluated 314 hard palate SCC and 411 upper alveolar ridge cases. They found a 13.65% prevalence of cervical metastasis and its correlation with larger tumor (4.1% for T1 to 24.7% for T4 tumors, $P < 0.001$). Extension of lymph node involvement was correlated to survival ($P < 0.001$).

We found a neck lymph node involvement rate of 24.4% and it has a trend associated with shorter survival ($P = 0.340$). The traditional concept has been that SCC of the hard palate and maxillary alveolus exhibits a low rate of occult metastasis^[7,31,32]. However, our results suggest that regional lymph node involvement is also frequent and relevant, and an elective treatment of the neck should be performed.

Regional recurrence rates in oral cancer have been described as between 30% and 47% in T1-2 carcinoma with untreated N0 neck, and they produce a significant decrease in patient survival. Some studies, including two prospective randomized trials, describe that neck recurrence rates decrease with the use of elective neck dissection^[22,33-35]. Regional recurrences in oral malignancies were associated with poor differentiation, larger tumor size, positive lymph node, and extracapsular involvement^[33,35]. A series of 114 cases with SCC of the maxillary alveolus and hard palate report regional recurrence rates of 26% in the N0 cohort ($n = 100$), and 35% of the patients had either initially N-positive neck or a later conversion from N0 to N-positive neck. Neck recurrence was associated with diminished overall survival but not with larger tumor size or postoperative radiation to the neck. Patients with initial diagnoses of N-positive and those who later developed neck recurrences had similar OS^[36].

Neck node recurrences occurred in 18 cases (23.1%) of our series and appeared at a mean time of 8.6 months; this likely represents occult metastases at presentation. Therefore, we had 42.3% of neck node involvement if we consider initial patients with positive nodes and N0 patients who developed neck recurrence. We also found that 25.9% of cases without clinical evidence of neck involvement developed recurrences at the neck. Neck recurrence had a trend to poor prognosis but did not achieve significance, probably because these cases received effective treatment including surgery (44.4%) or chemoradiation (11.1%).

Large tumor size, PNI, and LVI have been extensively associated with nodal metastasis and with shorter survival in HNSCC^[37,38]. Evaluation of classical prognostic factors in our series confirmed that larger tumors ($P = 0.019$), presence of PNI ($P = 0.039$), and LVI ($P = 0.021$) were associated with shorter OS, and LVI ($P = 0.026$) was associated with shorter DFS.

HNSCCs associated with smoking or drinking alcohol has been associated with a poor prognosis and are frequently located in laryngeal and hypopharyngeal cancer, respectively^[39]. Our analysis did not indicate higher prevalence rates of these carcinogen agents and did not find an association with prognosis in the upper maxilla.

Expression of p16 is a confident biomarker of HPV infection in OPSCC and both are associated with better outcome^[12,13,40-44]. In contrast to OPSCC, the rates of positive HPV in oral cancer are low, and recent studies suggest a disparity between the detection of HPV DNA and p16 expression when the prevalence of HPV is low^[45]. Evaluation of p16 staining in our series found that only 3 (7.4%) of upper maxilla SCC cases were considered positive for p16 staining. The p16-positive cases had a trend to be younger (48.7 vs. 63.7 years, $P = 0.067$), and all 3 cases were free of neck recurrence and alive at 5 years. This is the first time to our knowledge that p16 staining has been evaluated in upper maxilla SCC and could identify a group of patients with specific behavior. However, our analysis has the weakness of its small sample size and it needs to be confirmed by larger series (required size of series increases because of the low rates of p16-positive status in non-OPSCC).

The results of this retrospective analysis reveal that tumors of the hard palate and upper alveolus are associated with a high rate of neck node involvement and regional failure, which had a tendency to result in poor survival. Expression of p16 has a low rate in this pathology and could be associated with specific features.

DECLARATIONS

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Authors' contributions

Conception and design of the study and performed data analysis and interpretation: Salas E, Castaneda CA, Sanchez P, Postigo J

Performed data acquisition, as well as providing administrative, technical, and material support: Castillo M, Villegas V, Postigo J, Cano L, Casavilca S, Bernabe LA, Villa-Robles MR, Mantilla R, Belmar C, Guerra H

Drafted the article and made critical revisions related to the intellectual content of the manuscript, and approved the final version of the article to be published: all authors

Data source and availability

No additional data are available.

Financial support and sponsorship

None.

Conflicts of interest

There are no conflicts of interest.

Patient consent

Not applicable.

Ethics approval

The institutional review board approval was obtained from The Instituto Nacional de Enfermedades Neoplasicas (Lima, Peru).

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REFERENCES

1. Binahmed A, Nason RW, Hussain A, Abdoh AA, Sandor GK. Treatment outcomes in squamous cell carcinoma of the maxillary alveolus and palate: a population-based study. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2008;105:750-4.
2. Balasubramanian D, Ebrahimi A, Gupta R, Gao K, Elliott M, Palme CE, Clark JR. Tumour thickness as a predictor of nodal metastases in oral cancer: comparison between tongue and floor of mouth subsites. *Oral Oncol* 2014;50:1165-8.
3. Morris LG, Patel SG, Shah JP, Ganly I. High rates of regional failure in squamous cell carcinoma of the hard palate and maxillary alveolus. *Head Neck* 2011;33:824-30.
4. Ogura I, Kurabayashi T, Sasaki T, Amagasa T, Okada N, Kaneda T. Maxillary bone invasion by gingival carcinoma as an indicator of cervical metastasis. *Dentomaxillofac Radiol* 2003;32:291-4.
5. Simental AA Jr, Johnson JT, Myers EN. Cervical metastasis from squamous cell carcinoma of the maxillary alveolus and hard palate. *Laryngoscope* 2006;116:1682-4.
6. Montes DM, Schmidt BL. Oral maxillary squamous cell carcinoma: management of the clinically negative neck. *J Oral Maxillofac Surg* 2008;66:762-6.
7. Kruse AL, Gratz KW. Cervical metastases of squamous cell carcinoma of the maxilla: a retrospective study of 9 years. *Head Neck Oncol* 2009;1:28.
8. Mourouzis C, Pratt C, Brennan PA. Squamous cell carcinoma of the maxillary gingiva, alveolus, and hard palate: is there a need for elective neck dissection? *Br J Oral Maxillofac Surg* 2010;48:345-8.
9. Lin HW, Bhattacharyya N. Survival impact of nodal disease in hard palate and maxillary alveolus cancer. *Laryngoscope* 2009;119:312-5.
10. Li Q, Wu D, Liu WW, Li H, Liao WG, Zhang XR, Liu ZM, Guo ZM, Liu XK. Survival impact of cervical metastasis in squamous cell carcinoma of hard palate. *Oral Surg Oral Med Oral Pathol Oral Radiol* 2013;116:23-7.
11. Hashibe M, Brennan P, Benhamou S, Castellsague X, Chen C, Curado MP, Dal Maso L, Daudt AW, Fabianova E, Fernandez L, Wünsch-

- Filho V, Franceschi S, Hayes RB, Herrero R, Koifman S, La Vecchia C, Lazarus P, Levi F, Mates D, Matos E, Menezes A, Muscat J, Eluf-Neto J, Olshan AF, Rudnai P, Schwartz SM, Smith E, Sturgis EM, Szeszenia-Dabrowska N, Talamini R, Wei Q, Winn DM, Zaridze D, Zatonski W, Zhang ZF, Berthiller J, Boffetta P. Alcohol drinking in never users of tobacco, cigarette smoking in never drinkers, and the risk of head and neck cancer: pooled analysis in the International Head and Neck Cancer Epidemiology Consortium. *J Natl Cancer Inst* 2007;99:777-89.
12. Ang KK, Harris J, Wheeler R, Weber R, Rosenthal DI, Nguyen-Tân PF, Westra WH, Chung CH, Jordan RC, Lu C, Kim H, Axelrod R, Silverman CC, Redmond KP, Gillison ML. Human papillomavirus and survival of patients with oropharyngeal cancer. *N Engl J Med* 2010;363:24-35.
 13. Fakhry C, Westra WH, Li S, Cmelak A, Ridge JA, Pinto H, Forastiere A, Gillison ML. Improved survival of patients with human papillomavirus-positive head and neck squamous cell carcinoma in a prospective clinical trial. *J Natl Cancer Inst* 2008;100:261-9.
 14. Namazie A, Alavi S, Olopade OI, Pauletti G, Aghamohammadi N, Aghamohammadi M, Gornbein JA, Calcaterra TC, Slamon DJ, Wang MB, Srivatsan ES. Cyclin D1 amplification and p16(MTS1/CDK4I) deletion correlate with poor prognosis in head and neck tumors. *Laryngoscope* 2002;112:472-81.
 15. Weinberger PM, Yu Z, Haffty BG, Kowalski D, Harigopal M, Sasaki C, Rimm DL, Psyrri A. Prognostic significance of p16 protein levels in oropharyngeal squamous cell cancer. *Clin Cancer Res* 2004;10:5684-91.
 16. Lassen P, Eriksen JG, Hamilton-Dutoit S, Tramm T, Alsner J, Overgaard J. Effect of HPV-associated p16INK4A expression on response to radiotherapy and survival in squamous cell carcinoma of the head and neck. *J Clin Oncol* 2009;27:1992-8.
 17. Kreimer AR, Clifford GM, Boyle P, Franceschi S. Human papillomavirus types in head and neck squamous cell carcinomas worldwide: a systematic review. *Cancer Epidemiol Biomarkers Prev* 2005;14:467-75.
 18. Evans JF, Shah JP. Epidermoid carcinoma of the palate. *Am J Surg* 1981;142:451-5.
 19. Cady B, Catlin D. Epidermoid carcinoma of the gum. A 20-year survey. *Cancer* 1969;23:551-69.
 20. Hiratsuka H, Miyakawa A, Nakamori K, Kido Y, Sunakawa H, Kohama G. Multivariate analysis of occult lymph node metastasis as a prognostic indicator for patients with squamous cell carcinoma of the oral cavity. *Cancer* 1997;80:351-6.
 21. Wallwork BD, Anderson SR, Coman WB. Squamous cell carcinoma of the floor of the mouth: tumour thickness and the rate of cervical metastasis. *ANZ J Surg* 2007;77:761-4.
 22. Pitman KT. Rationale for elective neck dissection. *Am J Otolaryngol* 2000;21:31-7.
 23. Weiss MH, Harrison LB, Isaacs RS. Use of decision analysis in planning a management strategy for the stage N0 neck. *Arch Otolaryngol Head Neck Surg* 1994;120:699-702.
 24. Haddadin KJ, Soutar DS, Oliver RJ, Webster MH, Robertson AG, MacDonald DG. Improved survival for patients with clinically T1/T2, N0 tongue tumors undergoing a prophylactic neck dissection. *Head Neck* 1999;21:517-25.
 25. Yuen AP, Wei WI, Wong YM, Tang KC. Elective neck dissection versus observation in the treatment of early oral tongue carcinoma. *Head Neck* 1997;19:583-8.
 26. Capote A, Escorial V, Munoz-Guerra MF, Rodriguez-Campo FJ, Gamallo C, Naval L. Elective neck dissection in early-stage oral squamous cell carcinoma--does it influence recurrence and survival? *Head Neck* 2007;29:3-11.
 27. Janeway HH. The treatment of tumors of the superior maxilla. *Ann Surg* 1918;68:353.
 28. Martin H. Cancer of the gums (gingivae). *Am J Surg (Special Monograph)* 1941;54:770-806.
 29. Soo KC, Spiro RH, King W, Harvey W, Strong EW. Squamous carcinoma of the gums. *Am J Surg* 1988;156:281-5.
 30. Evans JF, Shah JP. Epidermoid carcinoma of the palate. *Am J Surg* 1981;142:451-5.
 31. Joe JK, Patel SG, Shaha AR. Management of the neck. In: Rhys Evans PH, Montgomery PQ, Gullane PJ, editors. Principles and Practices of Head and Neck Surgery and Oncology. London and New York: Taylor & Francis; 2003. p. 534-73.
 32. Sparano A, Weinstein G, Chalian A, Yodul M, Weber R. Multivariate predictors of occult neck metastasis in early oral tongue cancer. *Otolaryngol Head Neck Surg* 2004;131:472-6.
 33. Pentenero M, Gandolfo S, Carozzo M. Importance of tumor thickness and depth of invasion in nodal involvement and prognosis of oral squamous cell carcinoma: a review of the literature. *Head Neck* 2005;27:1080-91.
 34. Andersen PE, Cambroner E, Shaha AR, Shah JP. The extent of neck disease after regional failure during observation of the N0 neck. *Am J Surg* 1996;172:689-91.
 35. Jerjes W, Upile T, Petrie A, Riskalla A, Hamdoon Z, Vourvachis M, Karavidas K, Jay A, Sandison A, Thomas GJ, Kalavrezos N, Hopper C. Clinicopathological parameters, recurrence, locoregional and distant metastasis in 115 T1-T2 oral squamous cell carcinoma patients. *Head Neck Oncol* 2010;2:1.
 36. Os AD, Karakullukcu B, Leemans CR, Halmos GB, Roodenburg JL, Weert SV, Karagozoglu KH, Witjes MJ. Management of the clinically N0 neck in squamous cell carcinoma of the maxillary alveolus and hard palate. *Head Neck* 2016;38:1794-8.
 37. Massano J, Regateiro FS, Januario G, Ferreira A. Oral squamous cell carcinoma: review of prognostic and predictive factors. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2006;102:67-76.
 38. Jones HB, Sykes A, Bayman N, Sloan P, Swindell R, Patel M, Musgrove B. The impact of lymphovascular invasion on survival in oral carcinoma. *Oral Oncol* 2009;45:10-5.
 39. Sturgis EM, Cinciripini PM. Trends in head and neck cancer incidence in relation to smoking prevalence. *Cancer* 2007;110:1429-35.
 40. D'Souza G, Kreimer AR, Viscidi R, Pawlita M, Fakhry C, Koch WM, Westra WH, Gillison ML. Case-control study of human papillomavirus and oropharyngeal cancer. *N Engl J Med* 2007;356:1944-56.
 41. Worden FP, Kumar B, Lee JS, Wolf GT, Cordell KG, Taylor JM, Urba SG, Eisbruch A, Teknos TN, Chepeha DB, Prince ME, Tsien CI, D'Silva NJ, Yang K, Kurnit DM, Mason HL, Miller TH, Wallace NE, Bradford CR, Carey TE. Chemoselection as a strategy for organ preservation in advanced oropharynx cancer: response and survival positively associated with HPV16 copy number. *J Clin Oncol* 2008;26:3138-46.
 42. Begum S, Gillison ML, Ansari-Lari MA, Shah K, Westra WH. Detection of human papillomavirus in cervical lymph nodes: a highly

- effective strategy for localizing site of tumor origin. *Clin Cancer Res* 2003;9:6469-75.
43. Rischin D, Young RJ, Fisher R, Fox SB, Le QT, Peters LJ, Solomon B, Choi J, O'Sullivan B, Kenny LM, McArthur GA. Prognostic significance of p16INK4A and human papillomavirus in patients with oropharyngeal cancer treated on TROG 02.02 phase III trial. *J Clin Oncol* 2010;28:4142-8.
 44. Lassen P, Overgaard J, Eriksen JG. Expression of EGFR and HPV-associated p16 in oropharyngeal carcinoma: correlation and influence on prognosis after radiotherapy in the randomized DAHANCA 5 and 7 trials. *Radiother Oncol* 2013;108:489-94.
 45. Chung CH, Zhang Q, Kong CS, Harris J, Fertig EJ, Harari PM, Wang D, Redmond KP, Shenouda G, Trotti A, Raben D, Gillison ML, Jordan RC, Le QT. p16 protein expression and human papillomavirus status as prognostic biomarkers of nonoropharyngeal head and neck squamous cell carcinoma. *J Clin Oncol* 2014;32:3930-8.