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Original Article

Clinicopathological and immunohistochemical evaluation of oral and oropharyngeal squamous cell carcinoma in Chilean population

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Abstract: In oral and oropharyngeal squamous cell carcinoma (OCSCC and OPSCC) exist an association between clinical and histopathological parameters with cell proliferation, basal lamina, connective tissue degradation and surrounding stroma markers. We evaluated these associations in Chilean patients. A convenience sample of 37 cases of OCSCC (n=16) and OPSCC (n=21) was analyzed clinically (TNM, clinical stage) and histologically (WHO grade of differentiation, pattern of tumor invasion). We assessed the expression of p53, Ki67, HOXA1, HOXB7, type IV collagen (ColIV) and carcinoma-associated fibroblast (α-SMA-positive cells). Additionally we conducted a univariate/bivariate analysis to assess the relationship of these variables with survival rates. Males were mostly affected (56.2% OCSCC, 76.2% OPSCC). Patients were mainly diagnosed at III/IV clinical stages (68.8% OCSCC, 90.5% OPSCC) with a predominantly infiltrative pattern invasion (62.9% OCSCC, 57.1% OPSCC). Significant association between regional lymph nodes (N) and clinical stage with OCSCC-HOXB7 expression (Chi-Square test $P < 0.05$) was observed. In OPSCC a statistically significant association exists between p53, Ki67 with gender (Chi-Square test $P < 0.05$). In OCSCC and OPSCC was statistically significant association between ki67 with HOXA1, HOXB7, and between these last two antigens (Pearson's Correlation test $P < 0.05$). Furthermore OPSCC-p53 showed significant correlation when it was compared with α-SMA (Kendall's Tau-c test $P < 0.05$). Only OCSCC-pattern invasion and OPSCC-primary tumor (T) pattern resulted associated with survival at the end of the follow up period (Chi-Square Likelihood Ratio, $P < 0.05$). Clinical, histological and immunohistochemical features are similar to seen in other countries. Cancer proliferation markers were associated strongly from each other. Our sample highlights prognostic value of T and pattern of invasion, but the conclusions may be limited and should be considered with caution (small sample). Many cases were diagnosed in the advanced stages of the disease, which suggests that the diagnosis of OCSCC and OPSCC is made late.

Keywords: Squamous cell carcinoma, oral cavity cancer, oropharyngeal cancer, TNM, pattern of tumor invasion, p53, ki67, HOX genes, type IV collagen, carcinoma-associated fibroblast

Introduction

Oral cavity squamous cell carcinoma (OCSCC) is one of the six most common types of cancer in humans, with an annual incidence of 300,000 cases worldwide [1]. It represents 95% of all head and neck cancers, and with an increasing incidence over the last decade [2]. It is often diagnosed in advanced stages and the overall 5-year survival is below 50% [2], but early diagnosis is associated with high survival rates [3]. Tobacco and alcohol are present in 90% of cases [3], having both a synergetic effect [4]. Oropharyngeal squamous cell carcinoma (OPSCC) comprises tonsil and the base of tongue as well as the walls of the pharynx and

Table 1. Clinical and pathological characteristics of patients with OCSCC (n = 16) and OPSCC (n = 21)

Features	OCSCC n (%)	OPSCC n (%)
Gender		
Female	7 (43.8)	5 (23.8)
Male	9 (56.2)	16 (76.2)
TNM		
Primary tumor (T)		
T1	3 (18.8)	5 (23.8)
T2	5 (31.3)	5 (23.8)
T3	2 (12.5)	5 (23.8)
T4	6 (37.5)	6 (28.6)
Regional lymph nodes (N)		
0	8 (50)	6 (28.6)
1	4 (25)	5 (23.8)
2	3 (18.8)	8 (38.1)
3	1 (6.2)	2 (9.5)
Metastasis (M)		
M0	16 (100)	21 (100)
Clinical Stage		
I/II	5 (31.2)	2 (9.5)
III/IV	11 (68.8)	19 (90.5)
Differentiation degree		
Well	4 (25)	1 (4.8)
Moderate	8 (50)	17 (81)
Poor	4 (25)	3 (14.3)
Pattern of tumor invasion		
Cohesive	5 (37.1)	9 (42.9)
Infiltrative	11 (62.9)	12 (57.1)
Survival state		
Alive@	7 (43.8)	6 (31.6)
Deceased†	9 (56.2)	13 (68.4)*

@Percentage for those who remain alive until the end of the follow up period with specified feature. †Percentage of those who have died with the specified feature. *In survival of OPSCC patients 2 cases were excluded from the analysis.

the soft palate [5], shares the same risk factors that OCSCC, showing a subgroup associated to high-risk types of human papillomavirus (HPV), especially HPV16 [6]. In OCSCC and OPSCC exist an association between clinical and histopathological parameters with markers of cell proliferation, basal lamina and connective tissue degradation and surrounding stroma cells [7]. In order to study these associations, the aim of this study was to make a descriptive analysis in OCSCC/OPSCC Chilean patients, with focus in TNM, WHO grade of differentiation, proliferation, invasion, myofibroblasts and HOX genes markers. Additionally we conducted a univariate and bivariate analysis to assess the relationship between these variables with survival rates.

Table 2. Survival of patients with oral and oropharyngeal squamous cell carcinoma n (%)

State	Years survival		
	1	3	4
OCSCC			
Alive	15 (93.8)	8 (50)	8 (50)
Deceased	1 (6.3)	8 (50)	8 (50)
OPSCC			
Alive	14 (73.7)	10 (52.6)	7 (36.8)
Deceased	5 (26.3)	9 (47.4)	12 (63.2)

Material and methods

A retrospective analysis was made in files of Carlos Van Büren Hospital (Valparaíso, Chile) during the period from 2000 to 2010. All the specimens were incisional biopsies from patients with a diagnosis of OCSCC (n=16) and OPSCC (n=21), and new slides stained with hematoxylin and eosin were performed for a previous examination and histological description. Clinicopathological data, such as sex, site of the primary tumour, and clinical staging, were collected from patient's charts. The use of the samples and procedures were approved by the Hospital Scientific Committee and the Bioethics Committee of the University of Talca (number 2013-051, http://www.cesarrivera.cl/research/ethics051.pdf). Immunohistochemical techniques were executed in the Pathology Department, Faculty of Dentistry, University of Campinas, Piracicaba, Brazil, and the Department of Stomatology, University of Talca, Talca, Chile. In order to preserve confidentiality, data was encrypted.

Histopathological features

After the selection of incisional biopsy specimens from each case, new sections 5 μm thick were taken from the fixed paraffin blocks. The sections were stained with hematoxylin-eosin and were re-evaluated under light microscopy by two different observers independently, who did not know the clinical data (blind study).

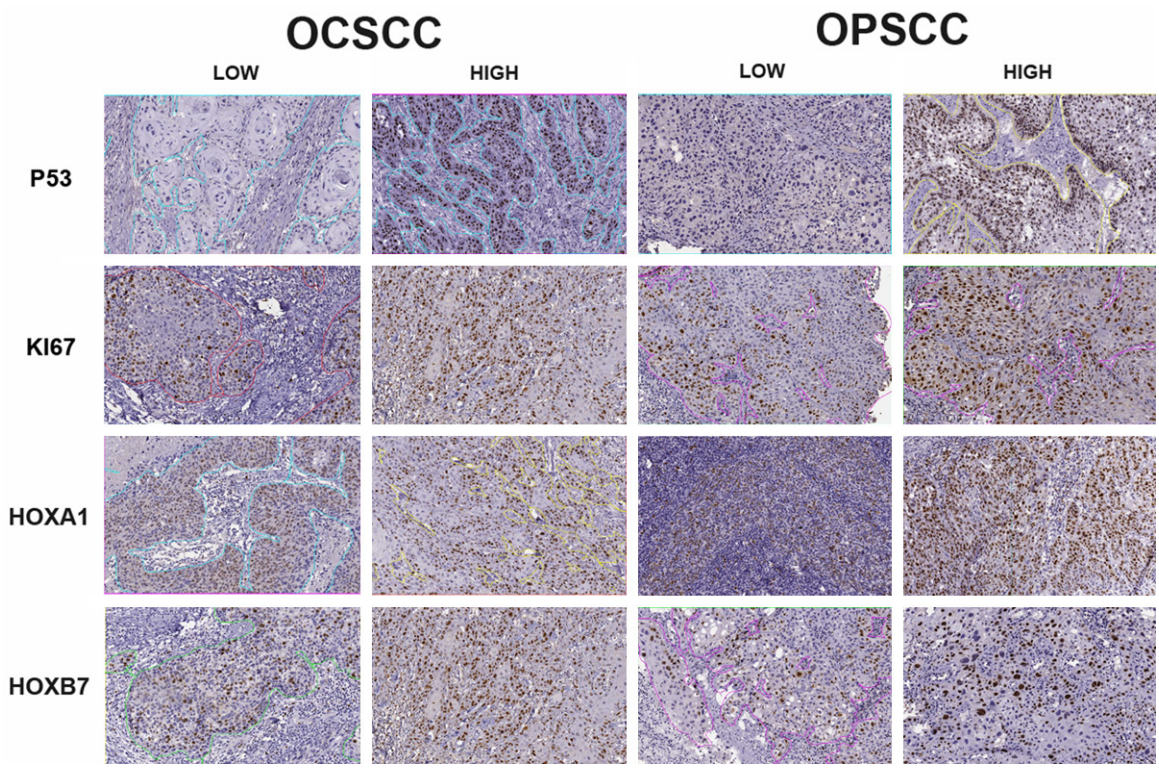

Figure 1. Aperio's IHC Nuclear Image Analysis. Rows show each antigen evaluated by Aperio. Columns correspond to values over and under of median. Sometimes it was necessary to defining the assessment area (in colors) for subsequent analysis.

Each specimen was histologically graded according to the International Classification of Tumors of WHO: well differentiated, moderately differentiated and poorly differentiated. The pattern of tumor invasion was analyzed according to the grading system proposed by Bryne [8], classifying it into cohesive (Bryne 1-2) and infiltrative (Bryne 3-4).

Immunohistochemistry

The reactions were conducted in 3 μm sections of the original formalin-fixed, paraffin-embedded tissues that were dewaxed with xylene and then hydrated in an ethanol series. The sections were treated with 3% H_2O_2 followed by antigen retrieval in 10 mM citrate buffer (pH 6.0) in a pressure cooker. After being washed in phosphate-buffered saline (pH 7.4), slides were incubated overnight with primary antibodies: anti-p53 (DO-7, Dako A/S, Denmark: 1/200), anti-Ki67 (MIB-1, Dako A/S, Denmark: 1/100), anti-HOXA1 (Polyclonal, Abcam, Cambridge, MA, USA 1/100) anti-HOXB7 (4C6, Abcam, Cambridge, USA: 1/100), anti-ColIV (CIV22; Bio SB, Santa Barbara, USA: 1/20), anti-α-SMA (1st 4; Dako A/S, Denmark: 1/100) followed by the LSAB method (LSAB + kit System-HRP, Dako). All slides were subsequently exposed to avidin-biotin complex and horseradish peroxidase reagents (LSAB kit; DakoCytomation, Glostrup, Denmark) and diaminobenzidine tetrahydrochloride (Sigma, St. Louis, MO) and subsequently counterstained with Carazzi hematoxylin. Adequate positive control sections were used for each antibody, and the negative control was obtained by omitting the primary specific antibody.

Immunohistochemical analysis

For quantitative analysis the stained slides were scanned using an Aperio ScanScope CS scanner (Aperio Technologies Inc., USA). The labeling index for p53, Ki-67, HOXA1 and HOXB7 was measured with the Aperio's IHC Nuclear Image Analysis algorithm (NIA). The NIA was split into two categorical groups (high and low), where the median was the cutoff point. ColIV staining was evaluated by two researchers, along the basement membrane of carcinomas and classified as continuous (C=a strip remains

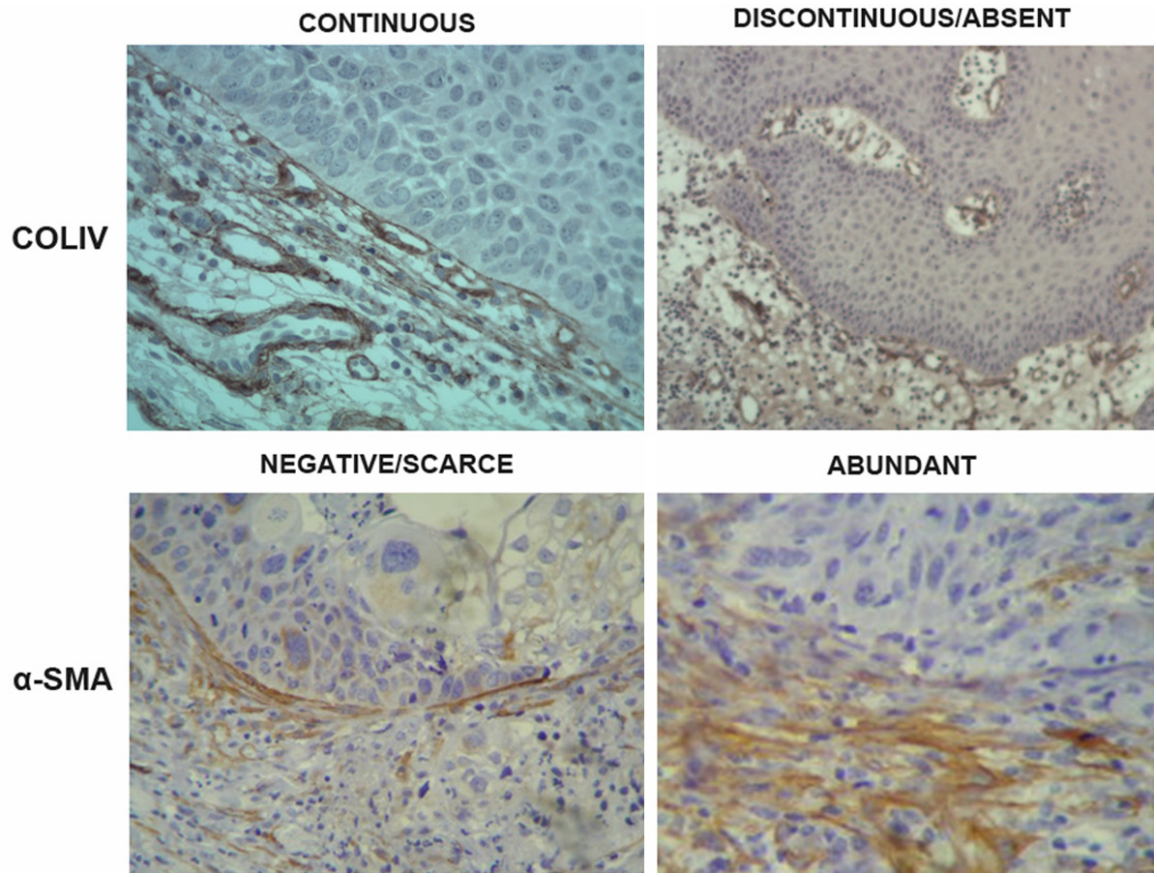

Figure 2. Expression of basal lamina degradation (OCSCC) and carcinoma-associated fibroblast (OPSCC) markers.

Table 3. Labeling index for each antigen

	OCSCC			OPSCC		
Antigen	NIA mean ± SD	NIA Median	Mode	NIA mean ± SD	NIA median	Mode
p53	29.1 ± 28	23.5	-	23.5 ± 27.8	6	-
ki67	41.4 ± 8.2	40.5	-	45.2 ± 13.8	47	-
HOXA1	36.2 ± 10.2	30.5	-	39.6 ± 10.8	38	-
HOXB7	41.9 ± 12.1	42	-	44.2 ± 11.5	43	-
ColIV	-	-	D/A (n=16)	-	-	D/A (n=20)
α-SMA	-	-	N/S (n=11)	-	-	N/S (n=14)

SD, standard deviation; D/A, Discontinuous + Absent; N/S, Negative + Scarce.

in the basal membrane), discontinuous (D=a fragmented membrane) and absent (A=when no staining was observed) [9], and then dichotomized (D/A and C). Presence of α-SMA was rated as negative + scarce (N/S) or abundant (A).

Statistical analysis

The interobserver concordance was evaluated through Lin coefficient for percentages and Kappa for categorized variables. The Kolmogorov-Smirnov test was used to test the hypothesis of normality. The relationship between the clinicopathological characteristics and survival status at follow-up was assessed using cross tables with Chi-Square Like-lihood Ratio (LR), Pearson's Correlation and Kendall's Tau-c test. Survival curves were created using the Kaplan Meier method with comparisons through the log rank test. In all the procedures a 95% confidence interval (CI) was used.

Table 4. Correlation between antigens expression and clinicopathological variables in oral and oropharyngeal squamous cell carcinomas

	OCSCC[#]					OPSCC					
Characteristic	p53	ki67	HOXA1	HOXB7	α-SMA	p53	ki67	HOXA1	HOXB7	COLIV	α-SMA
Clinic											
Gender	0.614	0.614	0.614	0.949	0.377	0.015*	0.696	0.027*	0.157	0.567	0.469
Primary tumor	1	0.317	1	0.614	0.59	0.835	0.505	0.256	0.123	0.283	0.537
Regional lymph nodes	0.317	0.317	0.003	0.012*	0.59	0.89	0.407	0.163	0.269	0.517	0.306
Clinical stage	1.06	0.59	1.06	0.049*	0.513	0.943	0.943	0.83	0.156	0.740	0.599
Histopathology											
Differentiation degree	1	1	1	0.771	0.755	0.283	0.283	0.237	0.283	0.819	0.469
Pattern of tumor invasion	0.59	0.59	0.59	0.838	0.611	0.130	0.256	0.899	0.801	0.375	1

[#]In OCSCC COLIV antigen was removed; no statistics are computed because COLIV is a constant. Features were correlated with by cross-tabulation and the Chi-Square test (only *p*-value is shown). *p ≤ 0.05.

Table 5. Correlation between antigens immunoexpression in oral and oropharyngeal squamous cell carcinomas

	OCSCC[#]					OPSCC					
Antigens	p53	ki67	HOXA1	HOXB7	α-SMA	p53	ki67	HOXA1	HOXB7	COLIV	α-SMA
p53											
Correlation	-	-0.151	-0.377	-0.006	0.174	-	-0.003	0.009	-0.128	-0.125	0.389
P-value	-	0.577	0.151	0.983	0.427	-	0.990	0.579	0.579	0.508	0.040*
Ki67											
Correlation	-0.006	-	0.749	0.801	0.125	-0.003	-	0.609	0.605	-0.204	-0.184
P-value	0.983	-	0.001	***	0.57	0.990	-	0.003**	0.019*	0.282	0.331
HOXA1											
Correlation	-0.151	0.801	-	0.809	-0.1	0.009	0.009	-	0.815	-0.203	-0.098
P-value	0.577	***	-	***	0.649	0.579	0.579	-	***	0.282	0.601
HOXB7											
Correlation	-0.006	0.801	0.809	-	-0.212	-0.128	0.609	0.815	-	-0.098	-0.098
P-value	0.983	***	***	-	0.335	0.579	0.003**	***	-	0.601	0.601
ColIV											
Correlation	-	-	-	-	-	-0.125	-0.204	-0.203	-0.093	-	0.158
P-value	-	-	-	-	-	0.508	0.282	0.282	0.620	-	0.480
α-SMA											
Correlation	0.174	0.125	-0.1	-0.212	-	0.389	-0.184	-0.021	-0.098	0.158	-
P-value	0.427	0.57	0.649	0.335	-	0.040*	0.331	0.911	0.601	0.480	-

[#]In OCSCC COLIV antigen was removed, no statistics are computed because COLIV is a constant. For comparision between numerical variables Pearson's Correlation was used. In numerical vs categorical variables Kendalls Tau-c was used. For comparision between categorical variables Kendalls Tau-b was used. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Results

Patients-age, sex, clinical stage and survival

Table 1 summarizes the main clinical and pathologic features observed. For all microscopic analysis, interobserver agreement (categorized variables) ranged from 75% to 97% among the examiners. Age of OCSCC patients ranged from 41 to 79 years with a median of 63.9 years for OCSCC patients and 60.4 for OPSCC. Males were mostly affected, with 56.2% in OCSCC and 76.2% in OPSCC. Patients were mainly diagnosed at III/IV clinical stages (68.8% OCSCC, 90.5% OPSCC) with a predominantly infiltrative pattern invasion (62.9% in OCSCC, 57.1% in OPSCC). Fifteen deaths from OPSCC group were registered and 2 not linked

Table 6. Association between survival status and clinical, pathological and immunohistochemical parameters of patients with OCSCC and OPSCC n (%)

	State							
	OCSCC				OPSCC			
Characteristic	Alive@ 7 (43.8)	Deceased# 9 (56.2)	LR	*P* value	Alive@ 6 (31.6)	Deceased# 13 (68.4)*	LR	*P* value
Clinic								
Gender			1.189	0.276			2.421	0.120
Female	2 (28.6)	5 (71.4)			3 (60)	2 (40)		
Male	5 (55.6)	4 (44.4)			3 (21.4)	11 (78.6)		
Primary tumor (T)			0.255	0.614			10.239	0.001**
T1/T2	4 (50)	4 (50)			6 (60)	4 (40)		
T3/T4	3 (37.5)	5 (62.5)			0	9 (100)		
Regional lymph nodes (N)			2.348	0.125			5.754	0,16
N0	5 (62.5)	3 (37.5)			0	6 (100)		
N+	2 (25)	6 (75)			6 (46.2)	7 (53.8)		
Clinical stage			0.779	0.377			1.624	0.202
I/II	3 (60)	2 (40)			0	2 (100)		
III/IV	4 (36.4)	7 (63.6)			6 (35.3)	11 (64.7)		
Histopathology								
Differentiation degree			0.84	0.772			0.784	0.376
Well	2 (50)	2 (50)			0	1 (100)		
Moderate/Poor	5 (41.7)	7 (58.3)			6 (33.3)	12 (66.7)		
Pattern of tumor invasion			4.035	0.045*			2.177	0.140
Cohesive	4 (80)	1 (20)			4 (50)	4 (50)		
Infiltrative	3 (27.3)	8 (72.7)			2 (18.2)	9 (81.8)		
Immunohistochemical								
p53			2.348	0.125			0.704	0.401
Low	5 (62.5)	3 (37.5)			4 (40)	6 (60)		
High	2 (25)	6 (75)			2 (22.2)	7 (77.8)		
Ki67			0.255	0.614			2.512	0.113
Low	4 (50)	4 (50)			5 (45.5)	6 (54.5)		
High	3 (37.5)	5 (62.5)			1 (12.5)	7 (87.5)		
HOXA1			2.348	0.125			2.512	0.113
Low	2 (25)	6 (75)			5 (45.5)	6 (54.5)		
High	5 (62.5)	3 (37.5)			1 (12.5)	7 (87.5)		
HOXB7			0.912	0.340			0.704	0.401
Low	3 (33.3)	6 (66.7)			4 (40)	6 (60)		
High	4 (57.1)	3 (42.9)			2 (22.2)	7 (77.8)		
ColIV			-	-				
Continuous	-	-			0	1 (100)	0.784	0.376
Discontinuous/Absent	7 (43.8)	9 (56.3)			6 (33.3)	12 (66.7)		
α-SMA			0.42	0.838				
Negative/Scarce	5 (45.5)	5 (54.5)			4 (33.3)	8 (66.7)	0.47	0.829
Abundant	2 (40)	4 (60)			2 (28.6)	5 (71.4)		

@Percentage for those who remain alive until the end of the follow up period with specified feature. #Percentage of those who have died with the specified feature. *In survival of OPSCC patients 2 cases were excluded from the analysis. RV Likelihood Ratio, as bigger the number is, more powerful is the marker. *$P < 0.05$, **$P < 0.01$.

to cancer (respiratory failure and brain-cranial trauma), which were excluded from the analysis. **Table 2** shows the survival for 1, 3, and 4 years after diagnosis.

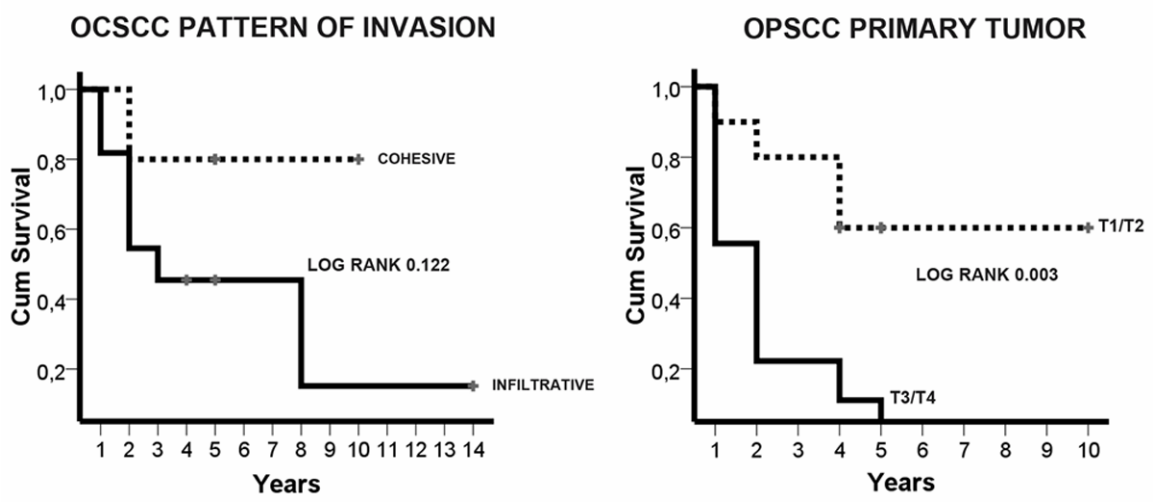

Figure 3. Kaplan-Meier curves for OCSCC-pattern of tumor invasion and OPSCC-primary tumor. Log Rank test shows significant differences between pairs, only in OPSCC.

Biomarkers expression and bivariate correlations

Figures 1 and **2** illustrate the immunohistochemical stains performed. Antibodies were labeled positivity in all samples tested. All antigen data were normally distributed (Kolmogorov-Smirnov). **Table 3** shows the median of the labeling index for each antibody. **Table 4** shows association between studied biomarkers with clinical and histopathological parameters of OCSCC and OPSCC. Highlight the significant association between regional lymph nodes (N) and clinical stage with OCSCC-HOXB7 expression (Chi-Square test $P < 0.05$). In OPSCC a statistically significant association exists between p53, Ki67 with gender (Chi-Square test $P < 0.05$). When the biomarkers correlation were evaluated (**Table 5**) in OCSCC and OPSCC the Pearson's Correlation test established significant association between ki67 with HOXA1, HOXB7, and between these last two antigens ($P < 0.05$). Additionally, in OPSCC, p53 showed significant correlation when it was compared with α-SMA (Kendall's Tau-c test $P < 0.05$).

Univariate analysis-survival state

The association between the immunohistochemical expression with survival was performed. In OSCCC patients pattern of tumor invasion resulted significantly associated with survival at the end of the follow up period (Chi-Square Likelihood Ratio, $P < 0.05$), where cases with infiltrative pattern corresponded to the 88.9% of all OCSCC deaths (**Table 6**). In OPSCC patients, primary tumor (T) has a significant association with survival (Chi-Square Likelihood Ratio, $P < 0.01$), T3/T4 cases represents the 69.2% of all OPSCC deaths (**Table 4**). All the immunohistochemical expression showed no association with survival.

Kaplan-Meier survival curves

Survival functions are presented in **Figure 3**. In the study of OCSCC invasion pattern, mean survival for cohesive group was 8.4 ± 1.4 years versus infiltrative, which was 5.5 ± 1.4 years, no showing significant differences (Log Rank test, $P=0.12$). Regarding the OPSCC primary tumor, median survival for T1/T2 group was 7.1 ± 1.2 years versus T3/T4, which was 2.1 ± 0.5 years, showing significant differences between the pairs (Log Rank test, $P=0.003$).

Discussion

OCSCC and OPSCC still represent a global health problem due of its impact and unpredictable behavior. They are tumors that occurs mainly in elderly people [3], however it has been reported an increase in the incidence of OPSCC associated to HPV infection in young patients [10]. In our sample, the mean age was the seventh decade for both OCSCC and OPSCC. No statistical association between the ages at diagnosis and survival was previously reported [3]. In our research, OCSCC and OPSCC affected mostly male, which is similar to

previous reports for OCSCC [3, 11] and OPSCC [12, 13]. Considering both groups (OCSCC/OPSCC), the mortality rate grouped by gender shows an increment in the women when compared with previously Chilean reports [14].

The correlation between HPV, and OPSCC was recognized by the International Agency for Research against Cancer (IARC) [5]. Furthermore, HPV-positive and HPV-negative OPSCC were suggested to likely be different entities [10]. We were not able to determine the presence of HPV in the sample, which is a limitation. Future research should consider this background, because the cases being HPV16-positive was shown to have a better clinical outcome compared to HPV-negative OPSCC [5].

The management of patients it is determined by health status, clinical staging system tumor-lymph node-metastasis (TNM classification) supplemented with additional pathological information [15]. In accordance with our findings, T was significantly associated with OPSCC survival status. From patients who died, 100% had tumors classified as T3/T4, also showing significant difference between T1/T2 versus T3/T4 group in survival curve. Therefore, a more severe classification for T in our sample is associated with poor survival. We were unable to find articles that inform T as a prognostic factor in OPSCC. In a previous research [16], from tumors of the oral cavity and oropharynx, it was demonstrated that the mean tumor thickness of patients who died with OCSCC was two times higher from those who died without this cancer.

Most patients of our sample (68.8% OCSCC, 90.5% OPSCC) were diagnosed in advanced clinical stages (stages III/IV), information consistent with the literature. Chilean National Institute of Cancer statistics shows similar data, as 78% of their OCSCC patients were classified as stages III and IV [17]. A previous research evaluated survival and patterns of recurrence in 200 oral/oropharyngeal mucosa cancers and shows that 72% of patients were diagnosed in advanced clinical stages III/IV [11].

The World Health Organization (WHO) recommends that the classification based on the degree of differentiation should be complemented by the evaluation of pattern of tumor invasion (PTI) [18]. In the clinical setting, the PTI is considered the most significant feature in terms of predictive values [3, 18]. PTI reflects malignant biological mechanisms such as the loss of contact inhibition and tumor cell motility. In this research, the PTI has also demonstrated an association with survival rate in OCSCC cases, and 88.9% of patients who died at the end of the follow-up showed an infiltrating pattern invasion. A recent study [19] reported that the PTI of OCSCC in clinical stage I and II is predictive for local and regional recurrence and disease-specific survival rate. Patients who had a pattern with tumoral satellites of ≥ 1 mm from the main tumor, showed a 42% probability of recurrence and 21% for death. Another study [20] stated that graduation for PTI was an independent prognostic factor in OCSCC cases and was significantly associated with distant metastasis and local recurrence; however there was no statistical correlation between PTI and other factors such as gender, age, tumor size, location and commitment of lymph nodes, clinical stage or degree of tumor differentiation, however a higher ranking for the PTI was analyzed and associated with poor survival rates and high probability of recurrence. The significant association between the PTI and OCSCC survival state in this study further supports this fact; however in the survival curves (Kaplan Meier), there was not statistically significant difference between the cohesive versus infiltrating type. The PTI did not decrease the OCSCC survival rates, probably because a larger sample size will be required for this parameter shows its influence.

In OCSCC and OPSCC different stromal cells and tumor cells activities were observed. An association between cell proliferation markers in the basal lamina and connective tissue has been identified, where hyperproliferative cancer cells may induce CoIIV degradation and facilitate tumor invasion, also stimulating an increase of cancer-associated fibroblasts [7]. Looking for immunohistochemical markers, which represent molecular events in the process of malignant transformation, proliferative markers (p53, Ki67, and HOXb7 HOXA1), basal membrane markers (COIIV) and tumor stroma myofibroblast markers (α-SMA) were studied.

Interestingly, interaction among p53, HOXA1, and gender was founded in our OPSCC patients, which may possibly be explained because head and neck cancer is traditionally a disease of elderly men with a history of chronic exposure to the carcinogenic effects of tobacco and alcohol [21].

In our study, the homeobox gene HOXB7 was significantly associated with regional lymph nodes commitment and clinical stage in OCSCC. Previous data suggest that HOXB7 may contribute to oral carcinogenesis by increasing tumor cell proliferation associated with TNM [22]. In OCSCC patients, a strongly positive linear correlation between Ki67, HOXA1 and HOXB7 was observed. Furthermore, in OPSCC group Ki67 showed a weak direct correlation versus HOXA1 and HOXB7 (these last two factors presents a strong positive correlation again). This evidence is consistent with recent findings, reporting that HOXA1 promotes cellular proliferation in vitro [23] and HOXB7 induces the accelerating G1/S transition, promote cell growth and proliferation [24].

The failure of applied immunohistochemistry in the analysis of molecular markers for predicting prognosis was unexpected. Selection of biomarkers in our research relied on molecules that previously demonstrated a potential prognostic role, and these showed no association with survival, which could be due to characteristics of our sample and storage of biological material in the hospital environment and its characteristics, that could affect antigen retrieval. One of the further difficulties of our design was to obtain a larger sample because there were missing data in clinical records, which not allow increasing the number of individuals.

Our results suggest that our patients, clinical, histological and immunohistochemical features are similar to seen in other countries. Many were diagnosed in the advanced stages of the disease, which suggests that the diagnosis of OCSCC and OPSCC is made late. The parameters associated with the survival state at the end of follow up were the OCSCC pattern of invasion and OPSCC maximum surface diameter of the primary tumor. Despite the fact that our patient material is small, we think it is important to explore the survival analysis, although the conclusions may be limited and should be considered with caution. The large battery of markers studied will be allowed to compare this data with future new lines of investigation.

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Disclosure of conflict of interest

None.

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