PROFILE OF LINE-1 METHYLATION STATUS IN OROPHARYNGEAL SQUAMOUS CELL CARCINOMA

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INTRODUCTION

Oropharyngeal cancer has increasing incidence in several continents. Oropharyngeal squamous cell carcinoma (OSCC) accounts for 90-95% of the tumor. In addition to the consumption of tobacco and alcohol, some Human Papillomavirus types have been associated with OSCC and in these cases are related to presentation and biological behavior. Hypomethylation of the Long Interspersed Nucleotide Element-1 (*LINE-1*) repetitive elements, a widely accepted surrogate of overall genomic DNA methylation content, was found to be associated with a poor prognosis in several cancers.

OBJECTIVE

To evaluate the profile of *LINE-1* methylation status in patients with OSCC diagnosed between 1999-2012.

METHODS

Records from 346 patients were collected, immunoexpression of p16 protein, qPCR for HPV16 *E6* and *LINE-1* methylation were performed, analyzed and submitted to bivariate (X^2 and Mann Whitney) and survival (Kaplan-Meier and Log rank) analysis.

RESULTS

White (48%) males (89.8%), aged between 41-60 years (59%), alcohol-drinkers (88.2%) and smokers (94.5%) were most affected. Predominated moderately-differentiated tumors (79.8%), clinical stage IV (54.9%) and treated with radiotherapy (56.9%). 84 were identified cases of disease progression, 106 cases of recurrence, mostly local (72.6%). 271 came to death, of these 256 patients had confirmed death from cancer. Negative cases were predominant for p16 immunoexpression (90.8%) and for *E6* HPV16 qPCR (88.1%). Regarding HPV status, only 21 cases (6.1%) were classified as HPV positive. LINE-1 methylation levels were largely heterogeneous (range, 16.39-73.95% and median, 54.33%) (**Table 1**).

Table 1: Sociodemographic, clinical-pathological and molecular features of patients (No=346).

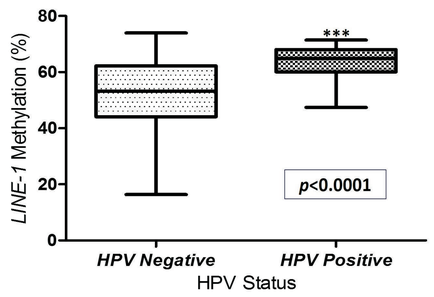
VARIABLE	CATEGORY	No. (%)
Gender	**Male**	**308 (89.8%)**
	Female	38 (11.2%)
Race	**White**	**166 (48.0%)**
	Brown	114 (32.9%)
	Black	66 (19.1%)
Age	≤40 years	8 (2.3%)
	41-60 years	**204 (59.0%)**
	>60 years	134 (38.7%)
Smokers	**Yes and ex**	**327 (94.5%)**
	No	19 (5.5%)
Alcohol-drinkers	**Yes and ex**	**305 (88.2%)**
	No	41 (11.8%)
Multiple tumor sites	One site	108 (31.2%)
	Two or more sites	**238 (68.8%)**
Initial treatment	Surgery	30 (8.7%)
	Radiotherapy	**197 (56.9%)**
	Radiotherapy and chemotherapy	68 (19.7%)
	Support	51 (14.7%)
Adjuvant treatment	Yes	27 (7.8%)
	No	**319 (92.2%)**
Clinical staging	I	26 (7.5%)
	II	43 (12.4%)
	III	87 (25.1%)
	IV	**190 (54.9%)**
Disease progression	Yes	84 (24.3%)
	No	**262 (75.7%)**
Recurrence	Yes	106 (30.6%)
	No	**240 (69.4%)**
Type of recurrence	**Local**	**77 (72.6%)**
	Regional	18 (17.0%)
	Distance	11 (10.4%)
Second primary tumor	Yes	17 (4.9%)
	No	**329 (95.1%)**
Death	**Yes**	**271 (78.3%)**
	No	75 (21.7%)
Death from cancer	**Yes**	**256 (97.0%)**
	No	8 (3.0%)
WHO grading	Well differentiated	12 (3.5%)
	Moderately differentiated	**276 (79.8%)**
	Poorly differentiated	58 (16.8%)
p16 immunoexpression	Positive	32 (9.2%)
	Negative	**314 (90.8%)**
E6 HPV16 qPCR	Positive	40 (11.9%)
	Negative	**295 (88.1%)**
HPV Status	Positive	21 (6.1%)
	Negative	**325 (93.9%)**
LINE-1 Methylation	Hypermethylation (63.41%)	20 (8.0%)
	Hypomethylation (52.64%)	**231 (92.0%)**

Association between advanced clinical stage and treated with radiotherapy (p<0.0001), smokers (p=0.013), disease progression (p=0.022), recurrence (p=0.008) and died (p=0.001) (**Table 2**).

Table 2: Distribution of socio-demographic and clinical-pathological variables according to clinical staging (No=346).

VARIABLE	CATEGORY	CLINICAL STAGING		
		Early (I + II) No. (%)	Advanced (III + IV) No. (%)	*p* value
Smokers	Yes and ex	69 (19.9%)	258 (74.6%)	**0.013**
	No	0 (0.0%)	19 (5.5%)	
Initial treatment	Surgery	18 (5.2%)	12 (3.5%)	**<0.0001**
	Radiotherapy	42 (12.1%)	155 (44.8%)	
	Radiotherapy and chemotherapy	7 (2.0%)	61 (17.6%)	
	Support	2 (0.6%)	49 (14.2%)	
Disease progression	Yes	10 (2.9%)	74 (21.4%)	**0.002**
	No	59 (17.1%)	203 (58.7%)	
Recurrence	Yes	30 (8.7%)	76 (22.0%)	**0.008**
	No	39 (11.3%)	201 (58.1%)	
Death	Yes	44 (12.7%)	277 (65.6%)	**0.001**
	No	25 (7.2%)	50 (14.5%)	

LINE-1 methylation level was significantly higher in HPV-positive OSCC patients than in HPV-negative ones (median, 63.41% and 52.64%, respectively; (p<0.0001) (**Graphic 1**).

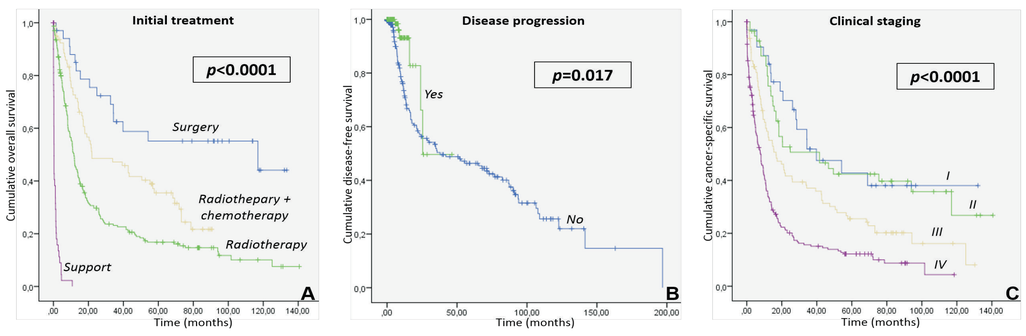


Graphic 1: Cumulative LINE-1 methylation index of HPV-negative OSCC (52.64%; n=231) and HPV-positive OSCC (63.41%; n=20) depicted in box plots (No=251).

Overall survival (OS) median was 28.39 months. Patients in clinical stage IV (p<0.0001), in supportive treatment (p<0.0001) (**Graphic 2A**) and with disease progression (p<0.0001) had a lower OS .

Already disease-free survival (DFS) was 22.96 months. Group with disease progression (p=0.017) (**Graphic 2B**), death (p<0.0001) and death from cancer (p=0.027) had a lower DFS.

Regarding the cancer-specific survival (CSS), clinical stage IV (p<0.0001) (**Graphic 2C**), supportive treatment (p<0.0001) and disease progression (p<0.0001) had a lower CSS.



Graphic 2: Kaplan-Meier curves. A - Overall survival; **B -** Disease-free survival; **C -** Cancer-specific survival.

CONCLUSIONS

- The profile of patients with OSCC analyzed doesn't differ from literature for developing countries.
- HPV-negative status associated with LINE-1 hypomethylation in OSCC.
- Habits, treatment, staging and death are important prognostic factors in this population, showing the aggressiveness of OSCC.

REFERENCES

CHATURVEDI, A. K. et al. Worldwide Trends in Incidende Rates for Oral Cavity and Oropharyngeal Cancers. J Clin Oncol, 18: 1-10, 2013.
EL-NAGGAR, A. K. et al. World health organization classification of tumours. WHO Classification of Head and Neck Tumors. Lyon: IARC Press, 2017. 133p.
EVANS, M. et al. Human Papillomavirus-associated oropharyngeal cancer: an observational study of diagnosis, prevalence and prognosis in a UK population. BMC Cancer, 13: 220-230, 2013.
FURLAN, C. et al. Prognostic significance of LINE-1 hypomethylation in oropharyngeal squamous cell carcinoma. Clin Epigenetics, 9: 1-11, 2017.
ZHAO, M. et al. Feasibility of quantitative PCR-based saliva rinse screening of HPV for head and neck cancer. Int J Cancer, 117: 605-610, 2005.

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