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Cell cycle markers and apoptotic proteins in oral tongue squamous cell carcinoma in young and elderly patients

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Abstract: The immunoexpression of p16, p53, and Bax in oral tongue squamous cell carcinoma (OTSCC) in young and elderly patients is assessed based on clinical and morphological parameters. The sample consists of 60 OTSCC cases: 30 in young (age ≤ 45 years) and 30 in elderly (age ≥ 60 years) patients. Clinical (tumor size, regional node metastasis, distant metastasis, and clinical stage) and morphological (histological grade of malignancy) parameters were evaluated. Immunohistochemical quantitative analysis was performed using anti-p16, anti-p53, and anti-Bax antibodies. None of the evaluated proteins exhibited statistically significant differences between young and elderly patients ($p>0.05$). There was a significant association of p16 immunoexpression with clinical parameters in elderly patients. There were no associations of p53 and Bax with any of the clinico-morphological parameters. Correlations between p16 and Bax and between p53 and Bax immunoexpression were observed in young patients ($r = 0.363$; $p = 0.048$) and in elderly patients ($r = 0.433$; $p = 0.017$), respectively. In conclusion, the assessed proteins could not be used to determine differences in the biological behavior of OTSCC between young and elderly patients. Therefore, all proteins activated the pro-apoptotic pathway of OTSCC in both groups.

Keywords: Cell Cycle; Apoptosis Regulatory Proteins; Young Adult.

Introduction

In recent years, there has been an increase in the overall incidence of oral tongue squamous cell carcinoma (OTSCC) in young patients[1,2,3] at a frequency that ranges from 0.13 to 12.76% among all cases of OTSCC.[2,3,4,5,6] Males are more frequently affected by OTSCC, regardless of age, although recent studies have indicated an increase in incidence among young women.[1,3] The etiopathogenesis of OTSCC in young patients is still widely discussed in the scientific literature because many patients are not exposed to the main risk factors, such as smoking and/or alcohol consumption.[1,3,4] Heredity[4] and human papillomavirus (HPV) infection have been suggested to play a role in this age group.[7]

Another point of discussion is that OTSCC sometimes has a more aggressive biological behavior among young patients,[8,9] whereas other studies show no difference in relation to elderly patients.[10,11,12] Thus, molecular studies on mechanisms of regulation and progression of OTSCC have been performed to elucidate differences in its biological behavior between young and elderly patients.[9,13]

It has been shown that cell cycle regulatory proteins, such as p16, encoded by the CDKN2A (9p21) gene and p53, encoded by TP53 (17p13.1), act on the etiopathogenesis[7] and biological behavior of OTSCC.[12] Overexpression of p53 protein has already been demonstrated in OTSCC in young and elderly patients, exhibiting significant difference for the former group.[14] Few studies have analyzed the immunoexpression of p16 and p53 in oral squamous cell carcinoma (OSCC), including oral tongue and other anatomical regions, in young and elderly patients.[7,12] Rushatamukayanunt et al.[7] observed statistical difference in p53 immunoexpression with higher scores in elderly patients. Galvis et al.[12] found no difference in p16 and p53 immunostaining between young and elderly patients.

Deregulation of the pro-apoptotic pathway, via Bax protein, which is a member of the BCL2 family,[15,16] is also related to progression of carcinogenesis. Overexpression of Bax has already been observed in OSCC,[16] but no study to date has specifically evaluated its immunoexpression in OTSCC between age groups, associating it with clinico-morphological parameters.

Therefore, the aim of the present study was to evaluate the immunostaining of p16, p53, and Bax proteins in a case series of OTSCC in young patients (age ≤45 years) and elderly patients (age ≥60 years), relating it to clinical and morphological parameters, to understand possible differences in the biological behavior of this tumor between age groups.

Methodology

Study design

Thirty cases of OTSCC in young patients (age ≤45 years) and 30 cases in elderly patients (age ≥60 years) were selected. The cases were diagnosed at three oncology referral hospitals in Brazil (Hospital Napoleão Laureano - João Pessoa/Paraíba, Hospital of Assistance Foundation of Paraíba - Campina Grande/Paraíba, and Hospital Araújo Gorge - Goiânia/Goiás) over a period of 14 years (from 2002 to 2016). Patients undergoing surgical treatment, with complete clinical and pathological data and with sufficient amounts of biological material for morphological and immunohistochemical analyses were included in the study. The cutoff age of 45 years was used following previously published recommendations.[5,6,11,14,17] Patients previously subjected to radiation therapy or chemotherapy were excluded. This study was approved by the Research Ethics Committee of Universidade Estadual da Paraíba (process no. 58218016.7.0000.5187).

Clinical data and morphological analysis

Clinical data such as gender, age, tumor size (T), regional lymph node metastasis (N), distant metastasis (M), and clinical stage (TNM) were obtained from the patient records. For morphological analysis, 5-µm sections were obtained from paraffin-embedded biological material. Histological sections were stained with hematoxylin and eosin and examined under a light microscope (Leica DM 500; Leica Microsystems Vertrieb GmbH, Wetzlar, Germany) by two previously trained examiners. Specimens were analyzed using the 2017 World Health Organization histological grading system, and tumors were classified into three types: well-differentiated, moderately differentiated, and poorly differentiated.

Immunohistochemical study

Three-micrometer sections were mounted on glass slides prepared with organosilane adhesive. Tissue sections were deparaffinized, rehydrated, and subjected to antigen retrieval. The sections were then immersed in 3% hydrogen peroxide to block endogenous peroxidase. After incubation with primary monoclonal anti-p16 (clone 1661; Santa Cruz Biotechnology, Dallas, TX; 1:200 dilution; 60 min), anti-p53 (clone DO-7; Dako, Carpinteria, CA; 1:250 dilution; 60 min), and anti-Bax antibodies (clone A3533; Dako, Carpinteria,

CA; 1:300; overnight), the sections were washed with Tris-HCl buffer and treated with a polymer-based complex (Reveal™; Spring Bioscience Corp., Pleasanton, USA). Peroxidase activity was visualized by immersing the sections in diaminobenzidine (DAB Substrate System; Spring Bioscience Corp., Pleasanton, USA). Finally, the histological sections were counterstained with Mayer's hematoxylin, dehydrated, and mounted on slides with a coverslip. Fragments of healthy oral mucosa were used as positive control for p16, OSCC tissue was used as positive control for p53, and tonsillar tissues were used as positive control for Bax. The negative control consisted of omission of primary antibodies in the protocol described above.

Immunohistochemical analysis

The histological sections were blindly analyzed by one previously trained examiner under a light microscope (Leica DM 500; Leica Microsystems Vertrieb GmbH, Wetzlar, Germany). Ten fields in OTSCC with high immunoreactivity were selected. Each field was photomicrographed (ICC 50HD; Leica Microsystems Vertrieb GmbH, Wetzlar, Germany) at 400× magnification, and images were transferred to ImageJ® software (Imaging Processing and Analysis in Java; National Institute of Mental Health, Bethesda, USA). Cells with brown-stained nuclei were considered positive for p16 and p53 immunoexpression and for cytoplasmic BAX immunoexpression. The positivity index (PI) was calculated (total of immunopositive neoplastic cells divided by 1,000 and multiplied by 100) for each case. This method for analysis of p16, p53, and BAX immunoexpression was adapted from Rushatamukayanunt et al.[7]

Statistical analysis

Results were analyzed using IBM SPSS Statistics 20.0 software (IBM Corp., Armonk, USA). Descriptive statistics was used for characterization of the sample. The chi-square test or Fisher's exact test was used to determine possible associations between clinico-morphological parameters and age groups. Analysis of the percentages of immunopositive cells for p16, p53, and Bax by the Kolmogorov-Smirnov test revealed absence of a normal distribution. Therefore, the nonparametric Mann-Whitney test was used to compare the median percentages of immunopositive cells for p16, p53, and Bax according to age groups. Spearman's correlation test was used to analyze the correlations among the immunoexpressions of p16, p53, and Bax in each group. The level of significance was set at 5% ($p < 0.05$) for all tests.

Results

Clinico-morphological study

Sixty OTSCC cases showed a higher prevalence among males in both young (age ≤ 45 years) (70.0%, 21/30) and elderly patients (age ≥ 60 years) (53.3%, 16/30), with a mean age of 38.17 ± 7.05 for young patients and of 70.60 ± 7.30 for elderly patients. In both groups, there was a high prevalence of small-sized tumors (T1/T2), corresponding to 63.3% in young patients and 80.0% in elderly patients. Similarly, absence of regional lymph node metastasis (N0) and distant metastasis (M0) was observed in most cases in both age groups. Regarding TNM staging, the elderly group showed a high frequency of early-stage (I/II) tumors (66.7%), whereas the young group showed identical rates for initial clinical stages I/II (50.0%) and for advanced stages III/IV (50.0%). The morphological analysis revealed a greater number of moderately and poorly differentiated tumors in both groups. Association of clinical and morphological parameters of OTSCC between young and elderly patients did not have statistical significance ($p > 0.05$) (Table 1).

Immunohistochemical analysis

Immunostaining demonstrated that p53 and p16 exhibited a brown staining pattern in the nucleus, while Bax showed the same pattern in the cytoplasm of neoplastic cells. P53 was mainly expressed at the periphery of tumor nests in young and elderly patients (Figures A and D, respectively). Immunostaining of p16 was predominantly observed at the periphery of tumor nests in young patients and diffusely stained in elderly patients (Figures B and E, respectively). Bax immunoexpression was

Table 1. Absolute and relative distribution of cases according to age groups and clinico-morphological parameters.

Clinico-morphological parameters	Groups		p-value
	Young patients	Elderly patients	
	n (%)	n (%)	
Sex			
Male	21 (70.0)	16 (53.3)	0.184*
Female	9 (30.0)	14 (46.7)	
Tumor size			
T1/ T2	19 (63.3)	24 (80.0)	0.152*
T3/ T4	11 (36.7)	6 (20.0)	
Regional metastasis			
N0	17 (56.7)	22 (73.3)	0.176*
N1–N3	13 (43.3)	8 (26.7)	
Distant metastasis			
M0	30 (100.0)	27 (90.0)	0.237**
M1	0 (0.0)	3 (10.0)	
Clinical stage			
I/ II	15 (50.0)	20 (66.7)	0.190*
III/ IV	15 (50.0)	10 (33.3)	
Histological grade of malignancy (WHO, 2017)			
Well-differentiated	13 (43.3)	10 (33.3)	0.426*
Moderately/poorly differentiated	17 (56.7)	20 (66.7)	

*Chi-square test; **Fisher's exact test

localized diffusely in the cytoplasm of tumor cells in both young and elderly patients (Figures C and F, respectively).

P53 immunoexpression was observed in 90.0% of cases in young (median 40.40) and in 97.0% in elderly (median 15.65) patients (p = 0.231). This immunoexpression was not significantly associated with any of the clinico-morphological parameters in the two age groups. P16 immunoexpression was found in 70.0% of OTSCC in young (median 9.80) and 97.0% in elderly (median 15.30) patients (p = 0.343), and there was a significant association with tumor size (p = 0.020), regional lymph node metastasis (p= 0.039), and clinical staging (p = 0.006) in elderly patients. Bax exhibited immunostaining in 100.0% of cases in both groups (p = 0.065) and there were no associations with any of the clinico-morphological parameters (Table 2).

Spearman's correlation test revealed a significant correlation between p16 and Bax immunoexpression in young patients (r = 0.363; p = 0.048) and between p53 and Bax in elderly patients (r = 0.433; p = 0.017).

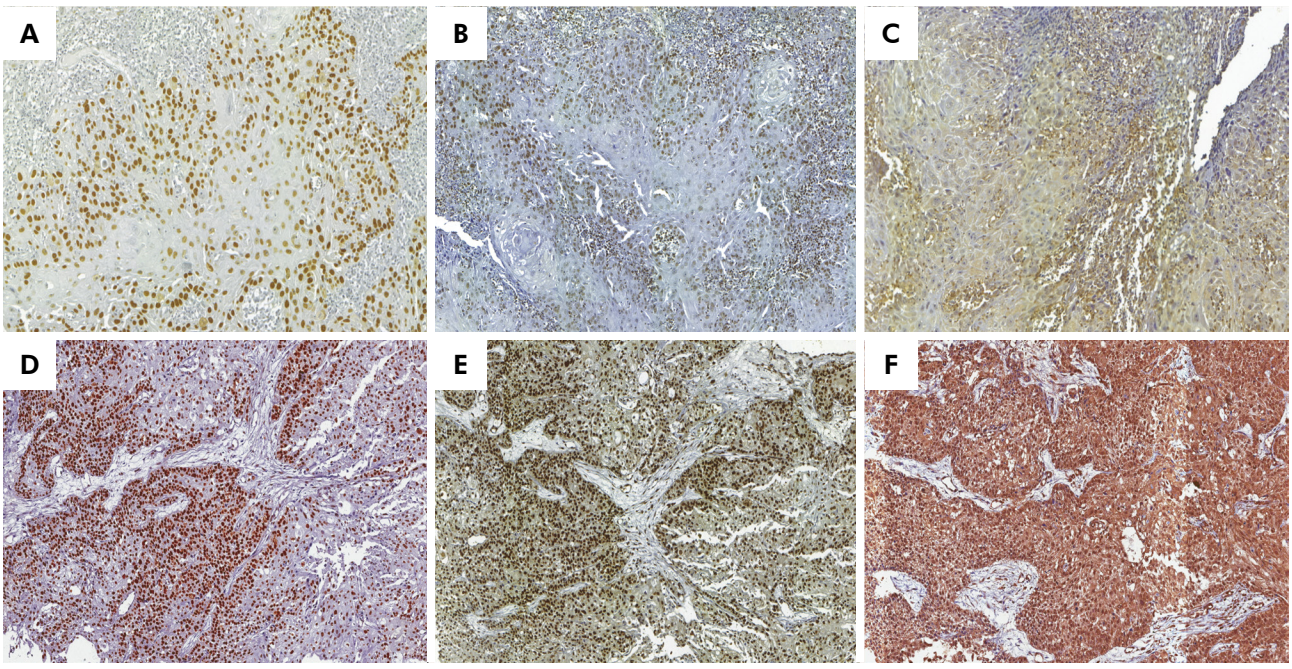

Figure. Immunohistochemical features of p53, p16, and Bax in OTSCC in young and elderly patients. (A) and (D) Nuclear immunoexpression of p53 mainly at the periphery of tumor nests in young and elderly patients, respectively (200x). (B) and (E) Nuclear positivity of p16 predominantly observed at the periphery of tumor nests in young patients and diffuse staining detected in elderly patients, respectively (200x). (C) and (F) Strong cytoplasmic immunopositivity for Bax localized diffusely in all tumor nests in young and elderly patients, respectively (200x).

Table 2. Comparison of immunoexpression of p16, p53, and BAX in cases of OTSCC in young (≤4 5 years) and elderly (≥ 60 years) patients according to clinico-morphological parameters.

Parameters	Groups	n	p16		p-value	p53		p-value	BAX		p-value
			Median	Q_{25}–Q_{75}		Median	Q_{25}–Q_{75}		Median	Q_{25}–Q_{75}	
Young patients											
Tumor size	T1–T2	19	9.30	0.40 – 34.40	0.586	37.30	1.00 – 52.40	0.355	53.10	31.00 – 73.80	0.533
	T3–T4	11	16.60	0.00 – 83.90		52.30	7.40 – 60.10		74.20	21.50 – 79.40	
Regional metastasis	N0	17	10.30	0.20 – 35.15	0.816	43.50	4.05 – 57.15	0.722	53.10	34.40 – 72.60	0.439
	N1–N3	13	6.90	0.00 – 74.15		16.10	3.60 – 57.70		74.50	12.20 – 87.25	
Clinical staging	I/ II	15	10.30	0.40 – 35.90	0.916	43.50	1.00 – 53.60	0.983	53.10	37.80 – 71.40	0.520
	III/ IV	15	6.90	0.00 – 64.40		21.40	5.70 – 59.40		74.20	12.40 – 83.20	
Histological grade of malignancy (WHO, 2017)	Well differentiated	13	0.00	0.00 – 33.75	0.065	36.10	0.75 – 46.20	0.149	44.80	15.20 – 70.45	0.075
	Moderately and poorly differentiated	17	16.60	1.00 – 50.90		52.30	6.55 – 60.40		71.40	37.50 – 85.55	
Older patients											
Tumor size	T1–T2	24	9.05	1.35 – 31.67	0.020	21.70	1.05 – 44.17	0.736	68.20	50.67 – 79.30	0.055
	T3–T4	6	50.05	26.70 – 74.95		3.80	0.60 – 61.55		84.60	66.32 – 88.40	
Regional metastasis	N0	22	8.55	1.25 – 32.40	0.039	15.65	0.95 – 36.75	0.412	70.95	56.65 – 83.40	0.851
	N1–N3	8	41.85	11.42 – 61.17		27.20	1.15 – 86.35		64.85	53.10 – 82.22	
Clinical staging	I/ II	20	6.80	1.15 – 31.15	0.006	21.70	1.05 – 37.45	0.912	69.75	52.15 – 79.45	0.481
	III/ IV	10	41.85	16.87 – 64.55		4.40	0.60 – 82.85		73.70	53.30 – 86.12	
Histological grade of malignancy (WHO, 2017)	Well differentiated	10	30.70	1.40 – 52.25	0.523	19.60	2.25 – 39.92	0.725	74.50	46.40 – 87.62	0.567
	Moderately and poorly differentiated	20	9.05	1.85–36.30		15.65	0.80–56.02		69.05	54.77–79.30	

Discussion

Possible differences in the biological behavior of OTSCC in young and elderly patients are still widely discussed.[8,9,12,17] Clinical[8,17] and molecular[7,9,11,12,18] aspects have been investigated as possible factors for this distinct behavior. Among these molecular aspects, cell cycle proteins (p16 and p53) and apoptotic proteins (Bax) play a crucial role in the progression of OTSCC, despite the paucity of studies on their participation in OTSCC in young and elderly patients.[7,12,19] Our findings demonstrated participation of p53, p16, and Bax proteins in the carcinogenesis of OTSCC, but no significant difference between age groups.

A matter of controversy surrounds the ranges established for classification of young age (≤ 30 years[8] to ≤ 60 years[20]), although OTSCC is more commonly observed at the ages of ≤ 40 years[2,10] and ≤ 45 years.[6,14,17] This lack of consensus hinders comparison with different studies. In the present study, patients aged ≤ 45 years were classified as young because this is the cutoff already established by our research group.[5,11]

In our study, p16 immunoexpression was associated with clinical parameters (larger tumors, regional lymph node metastasis, and advanced clinical stages) in elderly patients (age ≥ 60 years), thus suggesting that this protein may participate in later phases of OTSCC development in elderly patients, although there was no significant difference between the age groups, similar to what was found by Rushatamukayanunt et al.,[7] Farquhar et al.,[17] and Galvis et al.[12] Wang et al.[20] found no difference in p16 immunoexpression in OSCC between young (age ≤60 years) and elderly patients (age

> 60 years). Therefore, p16 immunostaining could not be utilized as a marker of distinct biological behavior of OTSCC between young and elderly patients.

The current study also did not observe a statistically significant difference in p53 immunoexpression between age groups, nor in the clinical parameters, similarly to other studies.[12,19] Goldstein and Irish,[21] in a literature review, showed absence of significant differences in the expression of p53, p21, Rb, and MDM2 proteins in younger patients, but a higher frequency of microsatellite instability in this age group. Santos-Silva *et al*.[9] observed a high incidence of abnormalities in the DNA ploidy of OTSCC in young patients, which indicates marked genomic instability and genetic differences in OTSCC between young and elderly patients. Rushatamukayanunt et al.[7] evaluated p16 and p53 immunoexpression in cases of OSCC in young (age ≤ 40 years) and elderly (age > 40 years) patients with presence and absence of HPV at high risk for malignancy and observed significant differences in p53 immunoexpression between age groups, association of combined immunoexpression of p16 and p53 with the histological grade of tumors, as well as absence of a relationship of these proteins with HPV. Those authors suggest that p53 immunoexpression has no correlation with HPV in OSCC in young and elderly patients.

In our study, there was no statistically significant difference in Bax immunoexpression in OTSCC between the age groups or in the clinico-morphological parameters. Likewise, Camisasca et al.[18] did not observe an association between Bax immunoexpression with clinicopathological parameters and with the survival of OSCC patients, regardless of age. Xie et al.[15] evaluated the immunoexpression of p53 and Bax in OTSCC and found no correlation with age groups, but they found that Bax overexpression was related to well-differentiated tumors and associated with an increase in survival. It was then suggested that Bax overexpression could indicate a better prognosis for OTSCC, despite the lack of difference in relation to age groups.

In our study, we found a positive correlation between p16 and Bax immunoexpression in young patients; therefore, the accumulation of p16 protein may be a pathway for the activation of pro-apoptotic factors in OTSCC in this age group. In elderly patients, there was correlation of p53 and Bax immunoexpression, which could indicate that accumulation of changes linked to p53 would still allow pro-apoptotic activation in OTSCC in this age group.

Limitations of this study are related to the absence of some data in patients' medical records, such as exposure to risk factors and disease-free survival.

In conclusion, we found that p16, p53, and Bax proteins play a role in OTSCC development, although they are not indicated for use as markers of differentiation of biological behavior of OTSCC in young and elderly patients. P16 plays a role in the pathogenesis of larger OTSCC in elderly patients, presence of regional metastasis, and later clinical stages of the disease, thus leading to a worse prognosis in this age group. Finally, the positive correlation between p16 and Bax immunoexpression in young patients and between p53 and Bax immunoexpression in elderly patients indicates that the pro-apoptotic pathway is mediated by the action of these proteins.

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References

1. Ng JH, Iyer NG, Tan MH, Edgren G. Changing epidemiology of oral squamous cell carcinoma of the tongue: A global study. Head Neck. 2017 Feb;39(2):297304. https://doi.org/10.1002/hed.24589
2. Mukdad L, Heineman TE, Alonso J, Badran KW, Kuan EC, St John MA. Oral tongue squamous cell carcinoma survival as stratified by age and sex: A surveillance, epidemiology, and end results analysis. Laryngoscope. 2019 Sep;129(9):207681. https://doi.org/10.1002/lary.27720

3. Xu Q, Wang C, Li B, Kim K, Li J, Mao M, et al. The impact of age on oral squamous cell carcinoma: A longitudinal cohort study of 2,782 patients. Oral Dis. 2019 Apr;25(3):73041. https://doi.org/10.1111/odi.13015
4. Hirota SK, Braga FP, Penha SS, Sugaya NN, Migliari DA. Risk factors for oral squamous cell carcinoma in young and older Brazilian patients: a comparative analysis. Med Oral Patol Oral Cir Bucal. 2008 Apr;13(4):E22731.
5. Santos HB, Santos TK, Paz AR, Cavalcanti YW, Nonaka CF, Godoy GP, et al. Clinical findings and risk factors to oral squamous cell carcinoma in young patients: A 12-year retrospective analysis. Med Oral Patol Oral Cir Bucal. 2016 Mar;21(2):e1516. https://doi.org/10.4317/medoral.20770
6. Ledesma-Montes C, Hernández-Guerrero JC, Durán-Padilla MA, Alcántara-Vázquez A. Squamous cell carcinoma of the tongue in patients older than 45 years. Braz Oral Res. 2018 Dec;32(0):e123. https://doi.org/10.1590/1807-3107bor-2018.vol32.0123
7. Rushatamukayanunt P, Morita K, Matsukawa S, Harada H, Shimamoto H, Tomioka H, et al. Lack of association between high-risk human papillomaviruses and oral squamous cell carcinoma in young Japanese patients. Asian Pac J Cancer Prev. 2014;15(10):413541. https://doi.org/10.7314/APJCP.2014.15.10.4135
8. Soudry E, Preis M, Hod R, Hamzany Y, Hadar T, Bahar G, et al. Squamous cell carcinoma of the oral tongue in patients younger than 30 years: clinicopathologic features and outcome. Clin Otolaryngol. 2010 Aug;35(4):30712. https://doi.org/10.1111/j.1749-4486.2010.02164.x
9. Santos-Silva AR, Ribeiro AC, Soubhia AM, Miyahara GI, Carlos R, Speight PM, et al. High incidences of DNA ploidy abnormalities in tongue squamous cell carcinoma of young patients: an international collaborative study. Histopathology. 2011 Jun;58(7):112735. https://doi.org/10.1111/j.1365-2559.2011.03863.x
10. Benevenuto TG, Nonaka CF, Pinto LP, Souza LB. Immunohistochemical comparative analysis of cell proliferation and angiogenic index in squamous cell carcinomas of the tongue between young and older patients. Appl Immunohistochem Mol Morphol. 2012 May;20(3):2917. https://doi.org/10.1097/PAI.0b013e31823277f6
11. Mesquita JA, Queiroz LM, Silveira ÉJ, Gordon-Nunez MA, Godoy GP, Nonaka CF, et al. Association of immunoexpression of the galectins-3 and -7 with histopathological and clinical parameters in oral squamous cell carcinoma in young patients. Eur Arch Otorhinolaryngol. 2016 Jan;273(1):23743. https://doi.org/10.1007/s00405-014-3439-y
12. Galvis MM, Santos-Silva AR, Jardim JF, Fonseca FP, Lopes MA, Almeida OP, et al. Different patterns of expression of cell cycle control and local invasion-related proteins in oral squamous cell carcinoma affecting young patients. J Oral Pathol Med. 2018 Jan;47(1):329. https://doi.org/10.1111/jop.12601
13. Costa SFS, Brennan PA, Gomez RS, Fregnani ER, Santos-Silva AR, Martins MD, et al. Molecular basis of oral squamous cell carcinoma in young patients: is it any different from older patients? J Oral Pathol Med. 2018 Jul;47(6):5416. https://doi.org/10.1111/jop.12642 PMID:28922483
14. Adduri R, Kotapalli V, Gupta NA, Gowrishankar S, Srinivasulu M, Ali MM, et al. P53 nuclear stabilization is associated with FHIT loss and younger age of onset in squamous cell carcinoma of oral tongue. BMC Clin Pathol. 2014 Aug;14(1):37. https://doi.org/10.1186/1472-6890-14-37
15. Xie X, Clausen OP, De Angelis P, Boysen M. The prognostic value of spontaneous apoptosis, Bax, Bcl-2, and p53 in oral squamous cell carcinoma of the tongue. Cancer. 1999 Sep;86(6):91320. https://doi.org/10.1002/(SICI)1097-0142(19990915)86:6<913::AID-CNCR4>3.0.CO;2-A
16. Zhang M, Zhang P, Zhang C, Sun J, Wang L, Li J, et al. Prognostic significance of Bcl-2 and Bax protein expression in the patients with oral squamous cell carcinoma. J Oral Pathol Med. 2009 Mar;38(3):30713. https://doi.org/10.1111/j.1600-0714.2008.00689.x
17. Farquhar DR, Tanner AM, Masood MM, Patel SR, Hackman TG, Olshan AF, et al. Oral tongue carcinoma among young patients: an analysis of risk factors and survival. Oral Oncol. 2018 Sep;84:711. https://doi.org/10.1016/j.oraloncology.2018.06.014
18. Camisasca DR, Honorato J, Bernardo V, Silva LE, Fonseca EC, Faria PA, et al. Expression of Bcl-2 family proteins and associated clinicopathologic factors predict survival outcome in patients with oral squamous cell carcinoma. Oral Oncol. 2009 Mar;45(3):22533. https://doi.org/10.1016/j.oraloncology.2008.05.021
19. Regezi JA, Dekker NP, McMillan A, Ramirez-Amador V, Meneses-Garcia A, Ruiz-Godoy Rivera LM, et al. p53, p21, Rb, and MDM2 proteins in tongue carcinoma from patients < 35 versus > 75 years. Oral Oncol. 1999 Jul;35(4):37983. https://doi.org/10.1016/S1368-8375(98)00126-2
20. Wang F, Zhang H, Xue Y, Wen J, Zhou J, Yang X, et al. A systematic investigation of the association between HPV and the clinicopathological parameters and prognosis of oral and oropharyngeal squamous cell carcinomas. Cancer Med. 2017 May;6(5):9107. https://doi.org/10.1002/cam4.1045
21. Goldstein DP, Irish JC. Head and neck squamous cell carcinoma in the young patient. Curr Opin Otolaryngol Head Neck Surg. 2005 Aug;13(4):20711. https://doi.org/10.1097/01.moo.0000170529.04759.4c