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Effect of p16 positivity in oral cavity and oropharyngeal squamous cell carcinoma

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Abstract

Objective: To determine the frequency of p16 positivity in oral cavity and oropharyngeal squamous cell carcinoma (OC/OP-SCC) and to reveal whether there is a difference between p16-positive and -negative cases according to clinicopathological parameters.

Methods: p16 antibody was retrospectively analyzed immunohistochemically in biopsies of 60 patients with OC/OP-SCC operated between 2007 to 2015. Comparison was performed for age, sex, smoking habit, alcohol consumption, site of the tumor, the level of keratinization, T stage, lymphovascular invasion, perineural invasion, recurrence of the tumor, and survival.

Results: Of the 60 patients (18 females, 42 males), the median was 58 (range: 27 to 75) years. Seventeen patients were p16-positive, and 43 patients were p16-negative. Comparison of p16-positive and p16-negative groups according to age, sex, T-stage, tumor subsite, tumor profundity, lymphovascular invasion, perineural invasion and survival was not statistically significant (p>0.05). We found statistical difference between two groups according to tumor recurrence, smoking habit, and the degree of keratinization.

Conclusion: In patients who underwent surgical treatment after the diagnosis of zOC/OP-SCC, p16 positivity may have a predictive role in terms of tumor recurrence.

Keywords: p16 positivity, oral cavity cancer, oropharyngeal cancer.

Özet: Oral kavite ve orofarengeal skuamöz hücreli kanserlerinde p16 pozitifliğinin etkisi

Amaç: Çalışmanın amacı oral kavite ve orofarengeal skuamöz hücreli karsinom (OK/OF SHK) hastalarında p-16 pozitifliğinin sıklığını belirlemek ve klinikopatolojik parametreler açısından p-16 pozitif ve p-16 negatif olgular arasındaki farklılığı ortaya koymaktır.

Yöntem: 2007 ila 2015 yılları arasında ameliyat edilmiş yassı epitel hücreli 60 OK/OF SHK hastasının biyopsilerinde immünohistokimyasal yöntemle p16 antikoru analiz edilmiştir. p16 pozitif ve p16 negatif hastalar yaş, cinsiyet, sigara içimi, alkol kullanımı, tümör yeri, keratinizasyon düzeyi, T evresi, lenfovasküler invazyon, perinöral invazyon, tümör nüksü ve retrospektif olarak sağkalım açısından karşılaştırılmışlardır.

Bulgular: Altmış hastanın (18 kadın, 42 erkek) ortanca yaşı 58 (aralık: 27–75) idi. On yedi hasta p16 pozitif ve 43 hasta p16 negatif idi. Yaş, cinsiyet, T evresi, tümör yerleşimi, tümör derinliği, lenfovasküler ve perinöral invazyon, ve sağkalım açısından gruplar arasında istatistiksel olarak anlamlı bir farklılık bulunmamaktaydı (p>0.05). Tümör nüksü, sigara içimi, ve keratinizasyonun derecesi açısından iki grup arasında istatistiksel farklılık mevcuttu.

Sonuç: Oral kavite ve orofarengeal skuamöz hücreli karsinom tanısı alarak cerrahi olarak tedavi edilen hastalarda p16 pozitifliği tümör nüksü açısından prediktif bir parametredir.

Anahtar sözcükler: p16 pozitifliği, oral kavite kanseri, orofarengeal kanser.

Head and neck squamous cell carcinoma is a frequent and global problem and oropharyngeal squamous cell carcinoma comprises about half of these tumors. The main predisposing factors linked with oral cavity and oropharyngeal squamous cell carcinoma (OC/OP-SCC) are alcohol consumption, tobacco use, and human papillomavirus (HPV) infection.[1]

In relevant publications, it has been suggested that molecular biomarkers such as p16, epidermal growth factor

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receptor (EGFR), B-cell lymphoma extra-large (Bcl-xL), p53, and Ki67 may have prognostic importance in OC/OP-SCC.[2]

The purpose of this study is to determine the frequency of p16 positivity in OC/OP-SCC and to investigate whether there is a difference between p16-positive and p16-negative cases with respect to prognostic factors and clinicopathologic parameters.

Materials and Methods

Study design

The study has been conducted in accordance with the principles of the Helsinki Declaration and approved by the local Institutional Review Board (05/02/2015, no:19).

A total of 60 patients with OC/OP cancer operated between 2007 to 2015 were analyzed.

p16 antibody was analyzed with immunohistochemistry. The histopathological classification and assessment of the tumors were conducted using light microscopy by one author. The patients with p16 positivity and negativity were retrospectively compared for age, sex, tobocco use, alcohol consumption, site of the tumor, the degree of keratinization, stage of the tumor, the profundity of the tumor, lymphovascular invasion, perineural invasion, tumor recurrence and survival.

Tumor tissue resected with negative margins and modified node dissection (level I–V) were performed in the patients with clinically positive for lymph node disease. Supraomohyoid node dissection (level I–III) were performed in clinically node-negative patients.

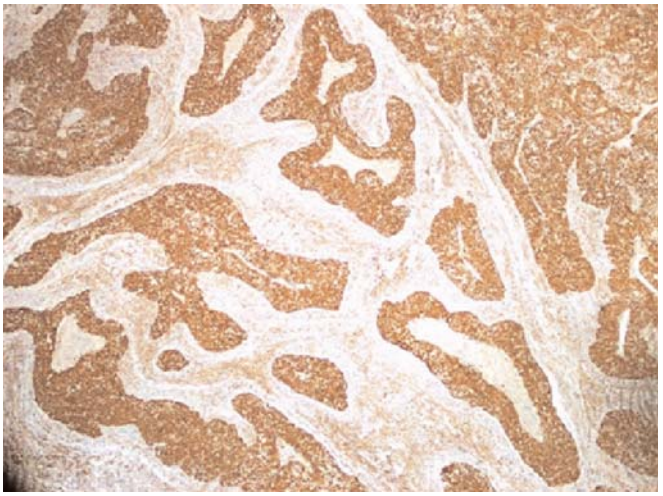

Fig. 1. Non-keratinizing squamous cell carcinoma. H&E ×10. (Keratinization in <25% tumor cells, and diffuse p16 staining).

Formalin-fixed paraffin-embedded samples were cut into 4-µm sections and stained with hematoxylin and eosin (H&E). Histological classification was made as non-keratinizing, moderate, and severely keratinizing squamous cell cancer (Figs. 1–3).

Sites of tumor were categorized as to tongue, tonsil and soft palate, the base of tongue and other regions. The

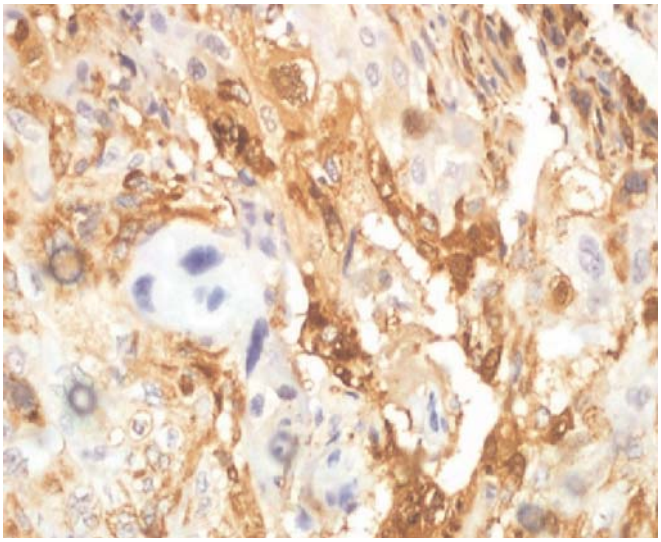

Fig. 2. Moderately keratinizing squamous cell carcinoma. H&E ×20 (Keratinization in 25–75% tumor cells, and moderate p16 staining).

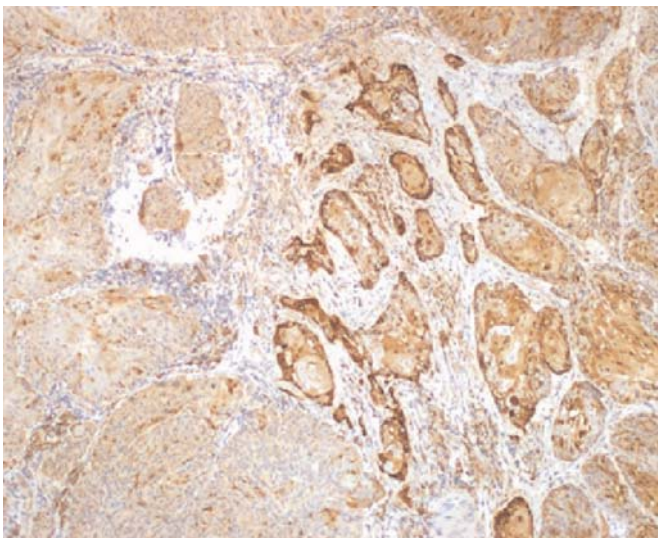

Fig. 3. Keratinizing squamous cell carcinoma. H&E x20 (Keratinization in >75% tumor cells, and weak p16 staining).

stages of tumor were T1 to T4. The profundity of tumor was classified as 0–10 mm, 10–20 mm, 20–30 mm.

Statistical analysis was carried out using Statistical Package for Social Sciences 21.0 (SPSS Inc., Chicago, IL, USA). Pearson's chi-square test was used to compare variables within groups. P<0.05 was considered significant.

Results

The median age of 60 patients (42 males, 18 females) was 58 (range: 27 to 75) years. Seventeen patients (28%) were p16-positive, while 43 patients (72%) were p16-negative.

Tumors were localized mostly in the tongue (n=23), followed by the tonsil and soft palate (n=10), base of the tongue (n=12), and other regions (n=15). At the time of admission, 38 patients were at early (T1 or T2) stage, and 22 patients were at late (T3 or T4) stage. Tumor profundity was 0–10 mm in 48 patients, 10–20 mm in 10 patients, 20–30 mm in two patients. Lymphovascular invasion was identified in 10 patients, perineural invasion in 14 patients and both in 5 patients. Median follow-up time was 24 months, and 25 (41%) patients died during follow-up.

Comparison of p16-positive and p16-negative groups according to clinicopathological parameters are presented in Table 1. Comparison of two groups according to age, sex, T-stage, tumor subsite, tumor profundity, lymphovascular invasion, perineural invasion and survival was not statistically significant (p>0.05). Among the study group, 31 (54%) patients were smokers and 11 (18%) were alcohol consumers. Four patients (23%) were smokers in p16-positive group, and 27 (62%) were smokers in p16-negative group. This result was statistically significant (p=0.03). Eleven patients showed local recurrence and all patients were in p16-negative group. This result was statistically significant (p=0.001). According to the degree of keratinization, histologic classification was non-keratinizing in 17 patients, moderately keratinizing in 17 patients, and severely keratinizing in 26 patients. In severely keratinizing group, 4 patients (23%) were p16-positive and 22 patients (41%) were p16-negative. This result was statistically significant (p=0.02). In moderately keratinizing group, eight patients (47%) were p16-positive and nine patients (20%) were p16-negative. This result was statistically significant (p=0.02).

Table 1. Comparison of p16-positive and p16-negative groups according to clinicopathological parameters.

		p16-positive group (n=17) n (%)	p16-negative group (n=43) n (%)	*p* value
Smokers (n=31)		4 (23%)	27 (62%)	0.03
Alcohol consumers (n=11)		2 (11%)	9 (20%)	-
Clinical T stage	T1-T2 (n=38)	10 (58%)	28 (65%)	>0.05
	T3-T4 (n=22)	7 (42%)	15 (35%)	>0.05
Tumor profundity	0-10 mm (n=48)	13 (76%)	35 (81%)	>0.05
	10-20mm (n=10)	4 (23%)	6 (13%)	>0.05
	20-30 mm (n=2)	0	2 (4%)	
Lymphovascular invasion		3 (17%)	7 (16%)	>0.05
Perineural invasion		5 (29%)	9 (20%)	>0.05
Site of tumor	Tongue (n=23)	7 (41%)	16 (37%)	>0.05
	Tonsil and soft palate (n=10)	4 (23%)	6 (13%)	>0.05
	Base of tongue (n=12)	4 (23%)	8 (18%)	>0.05
	Other (n=15)	2 (11%)	13 (30%)	>0.05
Keratinization	Non-keratinizing (n=17)	5 (29%)	12 (27%)	>0.05
	Moderately keratinizing (n=17)	8 (47%)	9 (20%)	0.03
	Severely keratinizing (n=26)	4 (23%)	22 (41%)	0.02
Survey (n=25)		8 (47%)	17 (39%)	>0.05
Recurrence	Local (n=11)	0	11 (25%)	
	Regional (n=8)	3 (17%)	5 (11%)	>0.05

Discussion

The human p16 protein, which is composed of 156 amino acids, was initially discovered in an in-vitro system to detect proteins that interact with human cyclin-dependent kinase 4.[3] The tumor suppressor function of p16 is associated with its capability to inhibit the catalytic activity of the cyclin-dependent kinase 4–6/cyclin D complex which is required for phosphorylation of retinoblastoma protein.[4]

In head and neck squamous cell carcinomas, three major mechanisms responsible for inactivation of p16 gene have been determined. These mechanisms are homozygous deletions, inactivation of mutation, and aberrant promoter methylation.[5] The frequency of absence of p16 protein expression in head and neck squamous cell carcinoma was found to be 74% (range: 55% to 90%) by immunohistochemical staining method.[6] In the present study, 17 patients (28%) were p16-positive, and 43 patients (72%) were p16-negative. These results are consistent with the relevant literature.

Ralli et al. reported that there was no significant difference between p16-positive and p16-negative groups according to age.[7] In the present study, we did not found any difference between groups with respect to age. Median age was 58 years in p16-negative group and 55 years in p16-positive group.

Smith et al. showed a statistically significant relationship between p16 expression, alcohol consumption, and tobacco use.[8] However, similar to our data, Lazarus et al. reported that there was no statistically significant association between p16 expression and tobacco use.[9] We found that four patients (23%) were smokers in p16-positive group, 27 patients (62%) were smokers in p16-negative group in our study. We did not compare two groups for alcohol consumption as the number of patients in groups was low for statistical comparison.

The most common tumor site was oropharynx in 63 cases (84%), especially from tonsils and base of the tongue. The highest incidence of p16 positivity was observed in tonsil tumors. However, no remarkable relationship was noted between p16-positivity and tumor site.[8] The most favorite site of the tumor was tongue in 23 patients in our study. There was no significant difference between 2 groups in terms of tumor site.

Ralli et al. reported that p16 over expression was more likely to occur in patients with higher histopathological grades.[7] The present study showed that non-keratinizing squamous cell carcinoma (NKSCC) is more likely to be in p16-positive group and these results are similar with the literature. Recent publications have demonstrated that HPV-positive oropharyngeal cancers occur at late stages (involvement of regional lymph nodes and distant metastasis) than HPV-negative cancers.[10] In the present study, contrary to the literature, we did not find any difference between p16-positive and p16-negative groups for tumor stage (early vs. late). In p16-positive group, 10 patients (58%) were at early stage (T1 and T2) and 7 patients (42%) were at late stage (T3 and T4); while in p16-negative group, 28 patients (65%) were at early stage and 15 patients (35%) were at late stage at the time of diagnosis.

Iyer et al. reported similar incidences for positivity of margins, lymphovascular invasion, and extracapsular spread in patients positive or negative for HPV.[11] In the current study, we found that lymphovascular invasion was present in 10 patients, perineural invasion was present in 14 patients, and both were present in five patients. We did not find any difference between two groups.

Advanced OPSCC patients with a solitary HPV-16 infection were 3 times more likely to develop distant metastases and were 2-3 times more likely to die earlier compared with HPV-negative patients.[12] In the present study, all patients with local recurrence were in p16-negative group. HPV-positive OC/OP cancers seem to be more sensitive to chemo-radiotherapy than HPV-negative tumors. This phenomenon is thought to result in improvement of progression-free and overall survival rates.[13] In contrast with the literature, our results on survival rate were not significant between p16-positive and p16-negative groups.

Conclusion

Our results indicated that p16 positivity was associated with tumor recurrence for patients with OC/OP-SCC and this finding supports the predictive role of p16 positivity, particularly in surgically treated patients.

Conflict of Interest: No conflicts declared.

References

1. Chandarana S, Lee J, Chanowski E, et al. Prevalence and predictive role of p16 and epidermal growth factor receptor in surgically treated oropharyngeal and oral cavity cancer. Head Neck 2013; 35:1083–90.
2. Lewis J, Thorstad W, Chernock R, Haughey B, Yip J, Zhang Q, et al. p16 positive oropharyngeal squamous cell carcinoma:an entity with a favorable prognosis regardless of tumor HPV status. Am J Surg Pathol 2010;34:1088–96.

3. Frisch M, Hjalgrim H, Jaeger AB, Biggar RJ. Changing patterns of tonsillar squamous cell carcinoma in the United States. Cancer Causes Control 2000;11:489–95.
4. Serrano M, Hannon GJ, Beach D. A new regulatory motif in cell-cycle control causing specific inhibition of cyclin D/CDK4. Nature 1993;366:704–7.
5. Rocco JW, Sidransky D. p16 (MTS-1/CDKN2/INK4a) in cancer progression. Exp Cell Res 2001;264:42–55.
6. Reed AL, Califano J, Cairns P, Westra WH, Jones RM, Koch W, et al. High frequency of p16 (CDKN2/MTS-1/INK4A) inactivation in head and neck squamous cell carcinoma. Cancer Res 1996;56:3630–3.
7. Ralli M, Singh S, Yadav S, Sharma N, Verma R, Sen R. Assessment and clinicopathological correlation of p16 expression in head and neck squamous cell carcinoma. J Cancer Res Ther 2016;12:232–7.
8. Smith EM, Rubenstein LM, Hoffman H, Haugen TH, Turek LP. Human papillomavirus, p16 and p53 expression associated with survival of head and neck cancer. Infect Agent Cancer 2010; 5:4.
9. Lazarus P, Sheikh SN, Ren Q, Schantz SP, Sten JC, Richie JP, et al. p53, but not p16 mutations in oral squamous cell carcinomas are associated with specific CYP1A1, and GSTM1 polymorphic genotypes and patient tobacco use. Carcinogenesis 1998;19:509–14.
10. Pintos J, Black MJ, Sadeghi N, Ghadirian P, Zeitoini AG, Viscidi RP et al. Human papillomavirus infection and oral cancer: a case control study in Montreal, Canada. Oral Oncol 2008;44:242–50.
11. Iyer N, Dogan S, Palmer F, Rahmati R, Nixon I, Lee N, et al. Detailed analysis of clinicopathologic factors demonstrate distinct difference in outcome and prognostic factors between surgically treated HPV-positive and negative oropharyngeal cancer. Ann Surg Oncol 2015;22:4411–21.
12. Lee LA, Huang C, Liao C, Lee LY, Hsueh C, Chen TC, et al. Human papillomavirus-16 infection in advanced oral cavity cancer patients is related to an increased risk of distant metastases and poor survival. Plos One 2012;7:1–11.
13. Chaturvedi AK, Engels EA, Anderson WF, Gillison ML. Incidence trends for human papillomavirus-related and -unrelated oral squamous cell carcinomas in the United States. J Clin Oncol 2008;26:612–9.

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