OTJHS
Online Turkish Journal of Health Sciences

e-ISSN: 2459-1467

OTSBD
Online Türk Sağlık Bilimleri Dergisi

Online Turkish Journal of Health Sciences 2022;7(2):196-201 *Online Türk Sağlık Bilimleri Dergisi 2022;7(2):196-201*

Baş-Boyun Skuamöz Hücreli Karsinomlarında EGFR, P16 ve Ki67 Ekspresyonunun Prognoza Etkisi

The Effect of EGFR, P16 and Ki67 Expression on Prognosis in Head and Neck Squamous Cell Carcinoma

[1]Sevil KARABAĞ, [1]Ayşegül İSAL ARSLAN, [2]Tolga ERSÖZLÜ

[1]Tekirdağ Namık Kemal University Medical Faculty, Pathology Department, Tekirdağ/TURKEY
[2]Tekirdağ Namık Kemal University Medical Faculty, Ear, Nose & Throat Department, Tekirdağ/TURKEY

Sevil Karabağ: https://orcid.org/0000-0002-8855-3798
Ayşegül İsal Arslan: https://orcid.org/0000-0001-8558-6378
Tolga Ersözlü: https://orcid.org/0000-0001-8629-6022

ÖZ

Amaç: EGFR ve HPV pozitif baş-boyun kanserlerinin, negatif olanlara kıyasla oldukça farklı klinik ve demografik özelliklerinden dolayı HPV ve EGFR ekspresyon durumu bağımsız prognostik faktör olarak kullanılabilir. Bu çalışmanın amacı baş-boyun skuamöz hücreli karsinom vakalarında EGFR, p16 ve ki67 ekspresyonlarının yaşam süresi ile ilişkisini saptamaktır.

Materyal ve Metot: Çalışmaya baş-boyun yerleşimli 47 orta derecede diferansiye skuamöz hücreli karsinom olgusu alındı. Olgulara immünohistokimya ile EGFR, p16 ve ki67 çalışıldı. Bu belirteçlerin yaşam süresiyle ilişkisi değerlendirildi.

Bulgular: Olguların 14'ü (%29,7) EGFR ekspresyonu göstermiş, 33'ünde (%70,3) boyanma görülmemiştir. Olguların 21'inde (%44,6) p16 ile boyanma görülmüş, 26'sında (%55,4) p16 ekspresyonu görülmemiştir. Hastaların ortalama takip süresi 32 aydır. 47 hastanın 15'i hastalık sebebiyle ex olmuştur. Yaşayan ve ex olan hastaların ki67 proliferasyon indeksi arasındaki fark istatistiksel olarak anlamlıdır (p=0,037). EGFR pozitif olan hastaların yaşam süresi, negatif olanlara göre anlamlı olarak daha kısadır (p=0,037). Ortalama sağkalım p16 pozitif 20 hastada 30 ay, p16 negatif hastalarda 33.5 ay idi (p=0,847).

Sonuç: Çalışmamız EGFR ve ki67'nin baş-boyun skuamöz hücreli karsinom hastalarında prognoz ve sağkalımı öngörmek için önemli bir belirteç olabileceğini desteklemektedir.

Anahtar Kelimeler: Baş-boyun skuamöz hücreli karsinom, EGFR, ki67, p16, prognoz

ABSTRACT

Objective: HPV and EGFR expression status may be utilized as an independent prognostic factor owing to the different clinical and demographic characteristics head and neck cancers. In the study, it was aimed to investigate the association between EGFR, p16 and ki67 expression and survival in patients with head and neck squamous cell carcinoma (SCC).

Materials and Methods: A total of 43 patients with SCC of the head and neck region were included in the study. EGFR, p16 and Ki67 were examined by means of immunohistochemistry. The association between these markers and survival was investigated.

Results: EGFR expression was detected in 14 cases (32.5%), Staining with p16 was positive in 20 cases (46.5%). Mean duration of follow up was 32 months. There was a statistically significant difference between ki67 proliferation indices of patients who survived and those who died (p=0.037). Survival was significantly shorter in EGFR positive patients compared to those negative for EGFR expressions (p=0.037). Mean survival was 30 months in the 20 p16 positive patients and 33.5 months in p16 negative patients (p=0.847).

Conclusion: This study supports that EGFR and Ki67 may be important markers to predict prognosis and survival in patients with head and neck SCC.

Keywords: Head and neck squamous cell carcinoma, EGFR, Ki67, p16, prognosis

Sorumlu Yazar / Corresponding Author:
Sevil Karabağ
Tekirdağ Namık Kemal University, Medical Faculty, Pathology Department Tekirdağ/TURKEY
Tel: +90532 1792484
E-mail: eesevil-krbg@hotmail.com

Yayın Bilgisi / Article Info:
Gönderi Tarihi/ Received: 06/10/2021
Kabul Tarihi/ Accepted: 28/02/2022
Online Yayın Tarihi/ Published: 01/06/2022

Atıf / Cited: Karabağ S and et al. The Effect of EGFR, P16 and Ki67 Expression on Prognosis in Head and Neck Squamous Cell Carcinoma. *Online Türk Sağlık Bilimleri Dergisi* 2022;7(2):196-201. doi: 10.26453/otjhs.1005416

INTRODUCTION

Head and neck squamous cell carcinoma (SCC) is the sixth most common malignancy worldwide and is associated with high morbidity and mortality.[1,2] Tobacco, alcohol and human papillomavirus (HPV) infection are known to be involved in the etiology of this carcinoma.[3] Despite the addition of chemotherapy (CT) and radiotherapy (RT) to surgical treatment, the five year survival rate remains at 50-66% in these patients.[4]

Activation of epidermal growth factor receptor (EGFR), a cell surface receptor member of the ErbB family, causes downregulation in PI3K-PTEN-AKT, MAPK, ERK and JAK/STAT pathways, triggering a phosphorylation cascade mediated by tyrosine kinases, thereby increasing cellular proliferation, invasion, angiogenesis and metastatic spread.[5,6]

Studies on EGFR expression and HPV analysis in head and neck SCC have shown that EGFR expression is higher in HPV positive cases than that in HPV negative cases, suggesting that viral oncoproteins may be associated with disruption of growth and differentiation signals.[7] The relationship of head and neck SCC with the EGFR pathway has increased the curative and palliative use of a monoclonal anti EGFR agent (cetuximab) as well as increasing research on other drugs that target EGFR, such as panitumumab, zalutumumab and nimotuzumab.[8-10]

Another poor prognosis factor in head and neck cancers, ki67, is a nuclear nonhistone protein that can be detected in actively proliferating normal and neoplastic cells. This protein reflects the growth rate of the tumor.[11,12]

The present study aims to determine EGFR, p16 and ki67 expression in cases with head and neck SCC, investigate survival in these patients and analyze the effect of these markers on survival and life expectancy. Thus, the aim is to identify more successful parameters in predicting the prognosis of patients and shed light on developing individualized treatment modalities.

MATERIALS AND METHODS

Ethical Status: The study was approved by the Tekirdag Namık Kemal University NonInterventional Clinical Trials Ethics Committee (Date: 20.04.2020, decision no:15). The study was carried out in accordance with international declaration, guideline.

A total of 43 cases diagnosed with SCC of the head and neck were enrolled in this study. Gender, age and tumor localization of the patients were recorded. Paraffin embedded tissues were retrieved from the archive, sections were taken samples were then introduced to a BenchMark XT device. Antibodies for EGFR (Ventana, RTU), p16 (ABM,1:100) and Ki67 (Ventana, RTU) were applied and staining was performed. Results were evaluated with an Olympus CX41 microscope by two pathologists.

Immunohistochemistry Evaluation: To evaluate EGFR, membranous staining pattern was scored on a scale of 0-3 as follows; 0: no staining, 1: weak incomplete staining in more than 10% of the tumor cells, 2: moderate staining in more than 10% of the tumor cells, 3: complete membranous staining in more than 10% of the tumor cells. Staining scores of 1, 2 and 3 were accepted as positive and 0 as negative during the statistical analysis.[13] To evaluate p16, strong and scattered brown staining in the nucleus and cytoplasm of 70% of the tumor cells was accepted as a p16 expression positive result.[14] Immunohistochemistry of ki67 was examined at x40 magnification. At least 1000 cells were counted, and percentage was calculated for each case.

Patients were followed up for a mean duration of 32 months in the MERNIS (Central Population Registration System) database for the survival analysis.

Statistical analysis: Patient demographics and data were analyzed using the SPSS 24 program (IBM, Chicago, Illinois, USA). Variables were expressed as frequency, percentage, mean (arithmetic mean, median), standard deviation (min-max), tables and graphs. Chi-square test was used to compare patient related variables in both groups, and Kaplan-Meier test was used for the survival analysis. $P<0.05$ was considered statistically significant.

RESULTS

All patients enrolled in this study were moderately differentiated SCC cases. Of the 43 patients, 28 were male and 15 were female. Mean age of the patients was 68.7 years (min: 41, max: 89). The tumor location was the lip in 14 cases, tongue in 13, oral mucosa in seven, nasopharynx in seven and tonsillar in two. Patients other than nasopharyngeal cancer underwent primary resection, lateral neck dissection including 1 and 4 levels, and / or elective neck dissection. Nasopharyngeal cancers were treated with stage 1 and 2 radiotherapy, while stage 3 cases were treated with chemoradiotherapy. The stages and localization of the cases and the treatments are presented in Table 1.

EGFR expression was detected in 14 cases (32.5%) while no staining was observed in 29 cases (67.5%). Since all cases with EGFR expression were either 2+ or 3+, they were evaluated together as positive cases during the statistical analysis.

Mean duration of follow up was 32 months (min: 4 months, max: 96 months). Of the 43 patients, 12 died because of their disease (min: 4 months, max: 72 months). Mean ki67 proliferation index was

43.6% in cases who died and 37.7% in survivors. There was a statistically significant difference between ki67 proliferation indices of patients who survived and those who died (p=0.037).

Mean survival was 36.1 months (±4.5) in EGFR negative patients and 22.1 months (±4.6) in EGFR positive patients. The difference between these figures was significant (p=0.037). The graph of EGFR and survival is presented in Figure 1a.

Staining with p16 was positive in 20 cases (46.5%) whereas there was no p16 expression in 23 (53.5%). Six cases showed staining both with p16 and EGFR while 18 were negative for EGFR and p16. There was no significant relationship between EGFR and p16 expressions (p=0.564).

Mean survival was 30 months (±5.9) in the 20 p16 positive patients and 33.5 months (±4.3) in p16 negative patients. The difference between these figures was not significant (p=0.847). The graph of p16 and survival is presented in Figure 1b.

p16 and EGFR analysis of the cases by gender is presented in Table 2. There was no significant difference between p16 and EGFR expressions according to localization of the tumors (p=0.739 and p=0.279).

Table 1. The stages and localization of the cases and the treatments.

Localization	Case Number	Stage	Treatment
Lip	14	I-II	Primer resection + elective neck dissection
Oral Tongue	13	I-II	Hemiglossectomy + lateral neck dissection (level1-4)
Floor of Mouth and Buccal Mucosa	7	I-II	Intraoral resection + lateral neck dissection (level1-4)
Tonsillar Fossae	2	I-II	Tonsillectomy + Ipsilateral elective neck dissection
Nasopharynx	7	I-III	Concurrent chemotherapy + Radiation

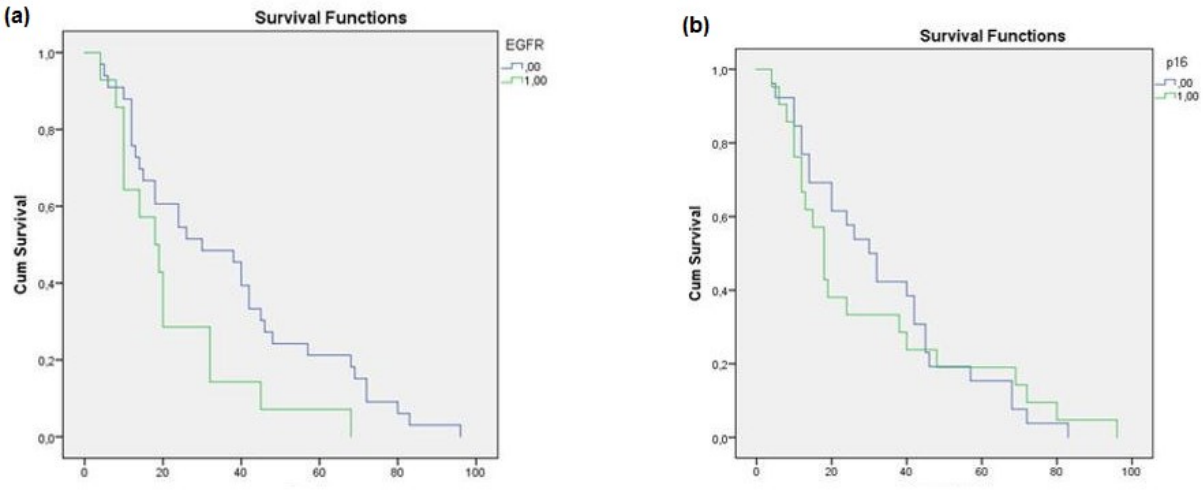

Figure 1. a, b. Association of EGFR (a) and p16 (b) expression and survival time (months).

Table 2. p16 and EGFR analysis of the cases by gender.

		Male	Female	p value
p16	negative	15	8	0.549
	positive	13	7	
EGFR	negative	20	9	0.238
	positive	8	6	

EGFR: Epidermal growth factor receptor; Chi-square test was used.

DISCUSSION AND CONCLUSION

EGFR expression, known to be involved in carcinomas such as lung and breast cancer, has also been investigated in various studies in head and neck SCC. Compared to normal mucosa, an increase in EGFR expression has been observed in dysplastic lesions and SCC.[13,15] In these cases, p53[6,16] and HPV[17] are the most studied biomarkers with known prognostic value. Studies support that p16 may be utilized as a clinical prognostic marker since p16 positive cases are associated with better response to treatment compared to p16 negative cases. Extensive HPV studies have paved the way for individualized therapeutic strategies.[6]

A study by Liu et al. investigating somatic mutations by means of cancer genome atlas analysis in 11314 patients across 32 different tumor types revealed an EGFR incidence of approximately 5% in head and neck SCC (10% of cases); however, most of these were identified as mutations of uncertain significance and only a minority were found to be pathogenic.[18]

In another study, Nagalakshimi et al were found the overall mutation rate of 75.19% in 129 head and neck SCC cases and 46% in 150 controls by PCR and single stranded confirmatory polymorphism techniques.[19]

In the present study, IHC revealed an EGFR expression rate of 32.5%. The data on the subject in the literature are quite controversial however, we believe this should be investigated further by means of more sensitive methods such as next generation sequencing and it should be elucidated to what extent these alterations are actually pathogenic.

Bossi et al. reviewed all relevant studies in the literature on EGFR protein expression, protein activation, gene copy numbers, polymorphism, mutation, EGFRvIII expression and EGFR ligand expression using cytogenetic, molecular and IHC methods in head and neck SCC. Based on the common conclusion of the studies included in this review, the authors concluded that EGFR expression detected by IHC would offer prognostic and predictive value and EGFR activation status may be used as a prognostic factor in patients undergoing surgery+ RT+ CT. For the other methods, they either found discordance across the studies or could not find sufficient data.[6]

Consistent with the literature, we identified a significant relationship between EGFR expression and survival in the present study and we believe EGFR may be utilized as a marker of poor prognosis in patients with head and neck SCC.

In a study where Kontic et al. investigated EGFR expression by means of IHC and HPV DNA by PCR in 196 cases, EGFR expression was identified particularly in subjects infected with low risk HPV. Longest survival durations were observed in subjects without HPV DNA and EGFR expression while shortest survival durations were in those who were positive both for HPV DNA and EGFR expression. Therefore, their study supports the notion that EGFR expression is a marker of poor prognosis.[13] Our study also confirms that EGFR expression is associated with poor prognosis, similar to the Kontic et al study.

There are several comparative studies in the literature investigating p16 and HPV PCR, which show that p16 is a reliable marker to detect HPV. These studies support a more favorable prognosis in HPV positive patients compared to negative cases due to the better response to treatment seen in the former group.[20]

Wang et al. applied p16 IHC in 93 cases with oropharyngeal SCC and 95 cases with oral SCC, revealing that the positivity rate of p16 was 25.8% and 9.5% in patients with oropharyngeal SCC and oral SCC, respectively. Overall survival in HPV positive patients with oropharyngeal SCC undergoing surgery or surgery + RT + CT was found to be significantly longer compared to HPV negative patients with oropharyngeal SCC (p=0.004). On the contrary, there was no statistically significant difference in survival of the patients with oral SCC (p=0.343). The authors showed that p16 status is a factor that affects prognosis in patients with oropharyngeal SCC while smoking index had an affect on prognosis in those with oral SCC, without an effect of p16 status on survival (p=0.237). They suggested that p16 may be used as a reliable marker to identify the HPV status.[21]

In our study, we applied p16 IHC to detect HPV. We observed p16 positivity in 46.5% of the cases included in the study. There was no significant association between p16 and survival. As a result, evaluating SCC in all localizations of the head and neck region, we observed no significant association between p16 expression and survival. We think that we can explain this situation with the small number of our cases and the inclusion of all head and neck tumors in the study instead of a specific region.

Maebayashi et al. investigated ki67 and p16 expression in terms of their association with treatment and prognosis, suggesting that the combination of ki67 expression and p16 analysis may provide a more reliable prognosis estimation compared to p16 expression status alone.[12] In the present study, we found a significant relationship between ki67 proliferation index and survival. This marker, which shows the growth rate of a tumor, may be used to estimate prognosis; however, we believe cut off values need to be determined through further detailed studies on this subject matter. The changes in tumor differentiation correlate with changes in ki67 proliferation index and the prognosis. We enrolled pa-

tients diagnosed with moderately differentiated SCC in order to rule out the differences in differentiation across tumors.

In conclusion we believe combined IHC analyses for ki67 and EGFR may be useful to predict the prognosis more successfully in patients with head and neck SCC. This study is the first in the literature to evaluate the relationship between p16, ki67 and EGFR IHC analysis to prognosis. The present study supports that EGFR and ki67 may be important markers in predicting the prognosis and survival as well as contributing to treatment guidance in patients with SCC of the head and neck. Moreover, we believe antiEGFR treatment may improve prognosis in these patients. Our study serves as a basis for further studies to be conducted on this subject.

Our study is limited because the sample size is small. We did not include larynx tumors because their HPV status and prognosis are quite different from other head and neck tumors. Our cases cover the head and neck SCC of the single center, excluding the larynx. Since the number of high and well-differentiated cases in our clinic is very low, only moderately differentiated cases were included in the study, and the relationship between tumor grade and markers could not be evaluated. We did not find a significant relationship between p16 negative and p16 positive patients in their mean survival time. This may be related to the fact that the study population did not explicitly include oropharyngeal cases but covered cases involving any location of the head and neck region.

Ethics Committee Approval: Our study was approved by the Tekirdag Namık Kemal University NonInterventional Clinical Trials Ethics Committee (Date: 20.04.2020, decision no:15). The study was carried out in accordance with international declaration, guideline.

Conflict of Interest: No conflict of interest was declared by the authors.

Author Contributions: Concept – SK, AA; Supervision – SK, AA, TE; Materials – SK, AA; Data Collection and/or Processing –SK, AA, TE; Analysis and/ or Interpretation – SK, TE; Writing –SK, AA, TE.

Peer-review: Externally peer-reviewed.

REFERENCES

1. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer. 2010;127:2893-2917.
2. Argyris PP, Slama Z, Malz C, et al. Intracellular calprotectin (S100A8/A9) controls epithelial differentiation and caspase-mediated cleavage of EGFR in head and neck squamous cell carcinoma. Oral Oncology. 2019;95:1-10.
3. Sivasithamparam J, Visk CA, Cohen EE, King AC. Modifiable risk behaviors in patients with head and neck cancer. Cancer. 2013;119:2419-2426.
4. Miller KD, Siegel RL, Lin CC, et al. Cancer treatment and survivorship statistics. CA Cancer J Clin. 2016;66:271-289.
5. Maxwell SA, Sacks PG, Gutterman JU, Gallick GE. Epidermal growth factor receptor protein-tyrosine kinase activity in human cell lines established from squamous carcinomas of the head and neck. Cancer Res. 1989;49(5):1130-1137.
6. Bossi P, Resteghini C, Paielli N, Licitra L, Pilotti S, Perrone F. Prognostic and predictive value of EGFR in head and neck squamous cell carcinoma. Oncotarget. 2016;7(45):74362-74379.
7. Almadori G, Cadoni G, Cattani P, et al. Human papillomavirus infection and epidermal growth factor receptor expression in primary laryngeal squamous cell carcinoma. Clin Cancer Res. 2001;7:3988-3993.
8. Vermorken JB, Mesia R, Rivera F, et al. Platinumbased chemotherapy plus cetuximab in head and neck cancer. N Engl J Med. 2008;359:1116-1127.
9. Mesía R, Henke M, Fortin A, et al. Chemoradiotherapy with or without panitumumab in patients with unresected, locally advanced squamous cell carcinoma of the head and neck (CONCERT-1): a randomised, controlled, open-label phase 2 trial. Lancet Oncol. 2015;16:208-220.
10. Giralt J, Trigo J, Nuyts S, et al. Panitumumab plus radiotherapy versus chemoradiotherapy in patients with unresected, locally advanced squamous cell carcinoma of the head and neck (CONCERT-2): a randomised, controlled, open-label phase 2 trial. Lancet Oncol. 2015;16:221-232.
11. Gioacchini FM, Alicandri-Ciufelli M, Magliulo G, Rubini C, Presutti L, Re M. The clinical relevance of Ki-67 expression in laryngeal squamous cell carcinoma. Eur Arch Otorhinolaryngol. 2015;272:1569-1576.
12. Maebayashi T, Ishibashi N, Aizawa T, et al. Roles of Ki-67 and p16 as biomarkers for unknown primary head and neck squamous cell carcinoma. European Archives of Oto-Rhino-Laryngology. 2019;276:1221-1229.
13. Kontic M, Colovic Z, Paladin I, Mirko Gabelica, Baric A, Pesutic-Pisac V. Association between EGFR expression and clinical outcome of laryngeal HPV squamous cell carcinoma. Acta Oto-Laryngologıca. 2019;139(10):913-917.
14. Oguejiofor KK, Hall JS, Mani N, et al. The prognostic significance of the biomarker p16 in oropharyngeal squamous cell carcinoma. clinical on-

cology. 2013;25:630-638.
15. Kontic M, Milovanovic J, Colovic Z, et al. Epidermal growth factor receptor (EGFR) expression in patients with laryngeal squamous cell carcinoma. Eur Arch Otorhinolaryngol. 2015;272:401-405.
16. Poeta ML, Manola J, Goldwasser MA, et al. TP53 mutations and survival in squamous-cell carcinoma of the head and neck. N Engl J Med. 2007;357:2552-2561.
17. Perrone F, Suardi S, Pastore E, et al. Molecular and cytogenetic subgroups of oropharyngeal squamous cell carcinoma. Clin Cancer Res. 2006;12:6643-6651.
18. Liu H, Zhang B, Sun Z. Spectrum of EGFR aberrations and potential clinical implications: insights from integrative pan-cancer analysis. Cancer Communications. 2020;40:43-59.
19. Nagalakshmi K, Jamil K, Rani PU. Polymorphism in EGFR gene and its association in head and neck cancer patients with tobacco and alcohol consuming habits. New Frontiers in Medicine and Medical Research. 2021;9:152-161.
20. Stanek L, Glendova K, Tesarova P, et al. Molecular and IHC analysis of head and neck carcinomas associated with HPV infection. Bratisl Med J. 2019;120(11):832-838.
21. Wang F, Zhang H, Xue Y, et al. A systematic investigation of the association between HPV and the clinicopathological parameters and prognosis of oral and oropharyngeal squamous cell carcinomas. Cancer Medicine. 2017;6(5):910-917.