Original Article

Ki-67, p-53, E-Cadherin, and β-Catenin Expression of Advanced Glotto-Subglottic and Supraglottic Larynx Carcinomas

A Kara, G Turan[1], M Guven, EM Guven[2], H Elden

Department of Otorhinolaryngology, Sakarya University Faculty of Medicine, Sakarya, Departments of [1]Pathology and [2]Anatomy, Kocaeli University Faculty of Medicine, Kocaeli, Turkey

Abstract

Background: Identifying tumor markers that can be used to determine the biological behavior of tumors and predicting their prognosis may be helpful in choosing treatment strategies. Besides the differences in the embryological and histological anatomy of the larynx in this regard, the possibility of molecular causes that can explain the different clinical behaviors has always been a question for the scientific world. **Aim:** In this study, we aimed to investigate whether there were any immunohistochemically molecular differences among laryngeal carcinoma cases originating from two different anatomical regions of the larynx. **Patients and Methods:** The study group consisted of 43 patients. The rate of supraglottic cancers was 41.8%, while the rest had glotto-subglottic tumors. Ki67, β-catenin, E-cadherin, and p53 were examined in pathology preparations obtained by laryngectomy surgeries. The data obtained were analyzed by comparing factors that may affect the prognosis of the disease and between tumors originating from the two different anatomical regions. **Results:** We did not see any statistically significant difference between groups for stage and grade of tumor, tumor recurrence rate, or lymphovascular or perineural invasion rated in terms of the investigated markers. In addition, there was no statistically significant difference between the two distinct groups in survival analysis. **Conclusions:** With these results, our study differs from some studies in the literature, and we think that this difference could be because the cases in our study consisted of advanced stage tumors and the groups investigated had similar survival rates.

Keywords: *β-catenin, E-cadherin, larynx cancer, p53, Ki67*

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Introduction

Laryngeal squamous cell carcinoma (LSCC) is the most common head and neck cancer in adults and accounts for 1-2% of all cancers.[1] Although the incidence of LSSC and the mortality rates have increased with time, the recovery rate is still higher than disease-related mortality. Regardless of the stage, treatment type, and tumor localization, the five-year survival rate, in general, is 60%.[2] However, the prognosis of supraglottic carcinomas has been shown to be worse than glottic tumors in many studies. This mainly occurs due to late diagnosis and early metastatic potential into the neck.[3] For these tumors, the five-year survival rates can decrease to 50%.[3]

TNM stage and tumor grade are the most important independent factors for prognosis and treatment decisions of head and neck tumors. However, it is clear that identifying tumor markers that can be used to determine the biological behavior of tumors and predicting their prognosis may be useful in choosing treatment strategies.[4] Today, it is possible by immunohistochemical examination to detect different kinds of proteins that are related to tumor cell

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Address for correspondence: Dr. H Elden, Department of Otorhinolaryngology, Sakarya University Faculty of Medicine, Adapazarı, Sakarya - 54000, Turkey.
E-mail: eldenhalil@gmail.com

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proliferation rate and invasive potential. Ki-67, one of the most used markers, is responsible for all active phases of the cell cycle except the G0 phase. Tumor protein P53 also functions in the cell cycle by blocking it in the G1 phase.[5] E-cadherin and β-catenin are involved in cell-cell adhesion, and downregulation of E-cadherin and expression of β-catenin have been associated with malignancies.[6]

In addition, the different clinical behaviors of laryngeal tumors belonging to different subdivisions of the larynx are also known as another clinical problem. Besides the differences in the embryological and histological anatomy of the larynx in this regard, the possibility of molecular causes that can explain the different clinical behaviors has always been a question for the scientific world. The number of studies on subdivisions of the larynx and molecular differences is less frequent in the current literature. In the present study, we aimed to evaluate the importance of Ki67, β-catenin, E-cadherin and p53 in terms of invasive and proliferative function and prognostic importance. In addition we compared these markers between advanced supraglottic and glotto-subglottic larynx carcinomas to determine if there was any difference in laryngeal subdivisions.

Materials and Methods

We analyzed tissue samples from 41 men and 2 women with laryngeal SCC (mean age 62.4 years, age range 44 – 88 years) at the Ear Nose Throat Department in Sakarya University Faculty of Medicine. The ethics committee of Sakarya University Faculty of Medicine approved the study (no: 71522473/050.01.04/100). We selected cases for inclusion among patients whose disease had been diagnosed in our department and for whom complete medical records were available. Informed consent was obtained from all of the participants.

After fixation of the specimens in 10% buffered formalin, they were dehydrated, embedded in paraffin blocks and four-micrometer thick sections were obtained for evaluation by light microscopy. Parameters such as degree of histological differentiation and lymphovascular and perineural invasion were examined with the samples.

For immunohistochemical staining investigations, polylysine-coated slides were prepared and studied with Ki-67 (Bond. Ki67 -MM1- Reference: PA0118, Leica Biosystems Newcastle Ltd), p53 (NCL-L-p53- D07, Leica Biosystems Newcastle Ltd), β–catenin (17C2; monoclonal, Reference PA0083, Leica Biosystems Newcastle Ltd), and E-cadherin (RTU-E-Cad, Leica Biosystems Newcastle Ltd) primary antibodies using the Leica Bond Maxautostainer (Leica Microsystems, Bannockburn, IL).

At least 1000 tumor cells were counted to determine the Ki-67 labeling index score and noted as a percentage according to the most stained area. Nuclear staining was graded from 0 to 3+. A score of 0 denoted non-stained samples, 1+ weakly stained (<10%), 2+ moderately stained (11%-50%) and 3 + strongly stained (>51%).

At least 1000 tumor cells were counted to determine p53 positive cells. If the nuclear positivity was more than 10% of the cells it was considered positive.

Another scoring system for β-catenin and E-cadherin assessment using both the intensity of immune staining and percentage of positive stained cells was used. Sections were graded for nuclear staining from 0 to 3+. A score of 0 indicated no staining or weak staining in less than 10% of tumor cells; 1+ indicated weak staining of 10-40% of cells or strong staining in less than 10% of tumor cells; 2+ indicated moderate staining of 40-70% of cells or strong staining in less than 10-40% of tumor cells; 3+ indicated moderate staining in more than 70% of cells or strong staining in less than 40-70% of tumor cells. Examples for the immune staining images can be seen in Figure 1.

Statistical analysis

The Statistical Package for the Social Sciences version 20.0 for Windows software program (IBM Corp., Armonk, NY, USA) was used to analyze the data. Non-parametric (independent-samples t-test,

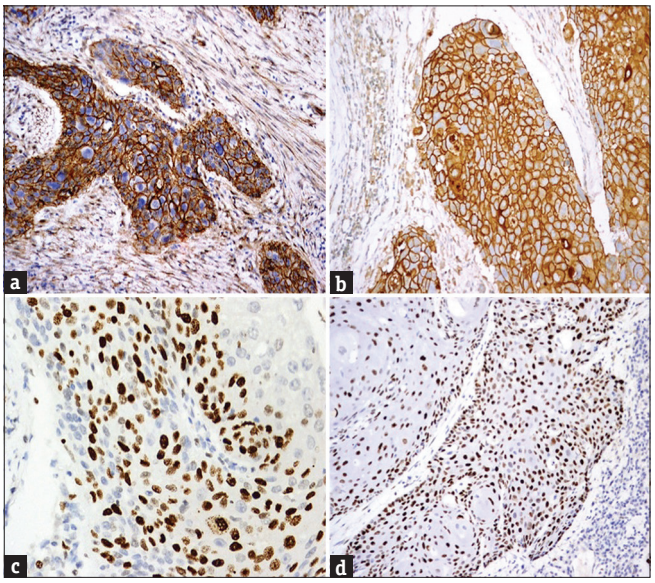

Figure 1: (a) Immunohistochemical staining for β-catenin expression (X20), (b) Immunohistochemical staining for e-cadherin expression (X20), (c) Immunohistochemical staining for Ki-67expression (X40), (d) Immunohistochemical staining for P53 expression (X20)

Chi-square test) tests were chosen according to normality distribution analysis (Shapiro–Wilk test). Results are shown as mean ± standard deviation, and a *P* value < 0.05 was accepted as significant.

Results

There were 43 patients with laryngeal SCC (18 patients in the supra-glottic carcinoma group and 25 patients in the glotto-subglottic carcinoma group) who were treated by partial or total laryngectomy surgeries. The average age of the patients was 62.4 ± 10.2 years; 64.1 ± 9.9 years in the supra-glottic carcinoma group and 61.3 ± 10.5 years in the glotto-subglottic carcinoma group. There was no statistically significant difference between the groups (p = 0.348). The distribution of cases according to demographic and clinical variables is shown in Table 1.

Table 1: Distribution of cases according to demographic and clinical variables (*n*=43)

Characteristic	All patients (%)
Age (years)	
Variation	44-88
Median	62.4
Average (standard deviation)	10.2
Gender	
Male	41 (95.3%)
Female	2 (4.7%)
Study groups	
Advanced supraglottic	18 (41.9%)
Advanced glotto-subglottic	25 (58.1%)
T stage	
T1	-
T2	-
T3	17 (39.5%)
T4	25 (60.5%)
N stage	
N0	30 (69.8%)
N1	2 (2.3%)
N2	11 (25.6%)
N3	1 (2.3%)
Degree of histological differentiation	
Well-differentiated	13 (30.2%)
Moderately differentiated	22 (51.2%)
Poorly differentiated	7 (16.3%)
Loco-regional recurrence	
No	31 (72.1%)
Yes	12 (27.9%)
Time for the occurrence of loco-regional recurrence (days)	
Variation	90-1479
Median	295
Average (standard deviation)	357.5

The P53 positivity rate was 44.4% in the supra-glottic carcinoma group and 56% in the glotto-subglottic carcinoma group (p: 0.455). In addition to this, there was not a statistically significant difference between groups for stage and grade of the tumor, tumor recurrence rate, and lymphovascular and perineural invasion rating [Table 2].

Moderate or strong Ki-67 staining was observed respectively in 11 (61.1%) and 7 (38.9%) patients in the supra-glottic larynx carcinoma group, and 14 (56%) and 10 (40%) patients in the glotto- subglottic larynx carcinoma group (p: 0.856). In addition to this, there was not a statistically significant difference between the groups for T stage, tumor grade, tumor recurrence rate, and lymphovascular and perineural invasion [Table 2].

There was no statistically significant difference between groups in terms of β-catenin cytoplasmic, β-catenin membranous, E-cadherin cytoplasmic or E-cadherin membranous staining [Table 2].

Table 2: Correlation of the expression of molecules under investigation, TNM stage, tumor's grade, lymphovascular invasion, and perineural invasion pattern with the location of the carcinoma

	Supraglottic	Glotto-subglottic	*P*
P53 positivity	8 (44%)	14 (56%)	0.455
Weak Ki-67 positivity	-	1 (4%)	
Moderate Ki-67 positivity	11 (61.1%)	14 (56%)	0.856
Strong Ki-67 positivity	7 (38.9%)	10 (40%)	
β-catenin cytoplasmic positivity	13 (72.2%)	21 (84%)	0.349
β-catenin membranous positivity	11 (61.1%)	19 (76%)	0.294
E-cadherin cytoplasmic positivity	15 (83.3%)	23 (92%)	0.382
E-cadherin membranous positivity	14 (77.8%)	18 (72%)	0.668
T stage			
T3	10 (55.6%)	7 (28%)	0.068
T4	8 (44.4%)	18 (72%)	
N stage			
N0	14 (77.8%)	16 (64%)	0.602
N1	0	1 (4%)	
N2	4 (22.2%)	7 (28%)	
N3	0	1 (4%)	
Grade			
1	4 (22.2%)	9 (36.0%)	0.208
2	9 (50%)	13 (52.0%)	
3	5 (27.8%)	2 (8.0%)	
Lymphovascular invasion	8 (44.4%)	10 (40.0%)	0.771
Perineural invasion	4 (22.2%)	7 (28.0%)	0.668
Recurrence	3 (16.7)	9 (36%)	0.163

Table 3: Correlation between staining of p53, Kİ 67, β-catenin cytoplasmic, β-catenin membranous, E-cadherin cytoplasmic, E-cadherin membranous Ki67 and Lymphovascular invasion, Perineural invasion, Recurrence status, T stage, and Grade (differentiation of the tumor)

	P					
	P53	Ki67	β-catenin cyt.	β-catenin memb.	E-cadherin cyt.	E-cadherin memb.
Lymphovascular invasion	0.009	0.381	0.560	0.707	0.929	0.779
Perineural invasion	0.255	0.450	0.795	0.313	0.432	0.514
Recurrence	0.146	0.595	0.214	0.052	0.675	0.405
T stage	0.151	0.609	0.669	0.925	0.071	0.480
Grade	0.184	0.241	0.831	0.136	0.326	0.391

cyt: cytoplasmic, memb: membranous

The recurrence rates of the diseases for supra-glottic and glotto-subglottic carcinoma groups were 16.7% and 36%, respectively. Kaplan–Meier survival analysis did not show a statistically significant difference between the groups (p: 0.997) [Table 2]. In addition, we did not find a statistically significant correlation between staining degree for suppressor gene p53, Ki 67, β-catenin cytoplasmic, β-catenin membranous, E-cadherin cytoplasmic, E-cadherin membranous and perineural invasion, recurrence status, tumor stage, lymph node metastases rates of tumor grade [Table 3].

Discussion

When the current published scientific literature is examined, it can be seen that there is increasing interest in the use of different molecular markers in predicting the prognosis and progression of laryngeal cancers.[3,6,7] One of the markers is Ki-67, a monoclonal anti-human antibody responsible for all active phases of the cell cycle except the G0 phase. Its role in the cell cycle is not yet clear. However, the most accepted hypothesis today is that it is a proliferation marker. Studies investigating the correlation of proliferation index, differentiation status, risk of recurrence, lymphatic metastasis, and survival rates of laryngeal tumors with Ki-67 levels have different results.[8-10] In a study published by Ashraf *et al*.,[11] the differences of Ki-67 and p53 levels in tumors that developed from different subdivisions of the larynx were investigated, as in the present study, and significant differences were observed in p53 levels. However, there was no significant difference between Ki-67 levels and tumor location. In another current study by Wachters *et al*.,[12] different molecules were examined in laryngeal subdivisions of patients with T1 and T2 LSCC, and they reported that Ki-67 levels were higher in supraglottic tumors. In the present study, we examined the correlation of Ki-67 immuno-staining results and tumor grade, stage, tumor localization, and perineural and lymphovascular invasion in a well-characterized series of patients with T3-T4 LSCCs. From our results we did not observe any significant association among these parameters, unlike the mentioned publications.

P53, which is another cellular molecule with an important mission in the cell cycle, blocks the cell cycle in the G1 phase with genotoxic stress. Disruption of pathways regulated by the p53 gene product leads to abnormal cell proliferation.[5] Although some studies showed a correlation between p53 and tumor progression, poorer survival rates, presence of lymph node metastasis, tumor stages, and the degree of histological malignancy, contradictory results have also been reported.[13,14] In a study published by Ashraf *et al*.,[11] the differences in p53 levels in tumors that developed from different subdivisions of the larynx were investigated, and a significant correlation was observed between p53 expression and tumor location. In the present study, we did not find a significant correlation between p53 expression and tumor grade, stage, tumor localization, and lymphovascular and perineural invasion, as some of the published studies did. However, we contradict the study conducted by Ashraf *et al*.[11] concerning tumor location and p53 levels. This difference may be related to their small study group size and abnormal patient distribution ratios regarding tumor location.

It is known that weaker β-catenin expression is related to aggressiveness of head and neck and nasopharyngeal carcinomas.[15,16] In the study presented by Ruiz *et al*.,[3] it was noted that β-catenin in supraglottic LSCC was located in the membrane and cytoplasm. However, β-catenin was found in the membranous location in most of glottic LSCCs. In a similar study by Goiliomus *et al*., they documented a statistically significant variation in the expression of membranous β-catenin between glottic and supra-glottic carcinomas in favor of the glottic location. However, there was no statistically significant difference in nuclear β-catenin levels.[6] In the study of Nardi *et al*. and Wachters *et al*. there was no such association for β-catenin expression.[17] In the present study, we did not document a statistically significant difference between the groups, in both membranous and

cytoplasmic β-catenin levels. From the contradictory information in the literature and our findings in the present study, we think that β-catenin levels are not important either in tumor localization or prognostic terms.

In the literature, the expression of E-cadherin has been investigated extensively for larynx tumors. Lower levels are associated with poor differentiation of the tumor, risk of nodal metastases, TNM stages, shorter disease-free survival, and less differentiated tumor grade by different authors.[17,18] In contrast to these results, Greco *et al*.[19] observed that patients whose tumors overexpressed both cytoplasmic and membranous E-cadherin experienced worse three-year overall survival. In different studies presented by Ahmed *et al*.[18] and Nardi *et al*.,[20] they documented a statistically significant variation in the expression of E cadherin between glottic and supra-glottic carcinomas in favor of glottic location. However, in the present study, there was no statistically significant difference between the groups in both the membranous and cytoplasmic E-cadherin levels, and we did not document a statistically significant difference in terms of both tumor aggressiveness or prognosis.

Conclusion

We did not find a statistically significant variation in the expression of the investigated parameters between glotto-subglottic and supra-glottic larynx carcinomas. These rates correlate with similar five-year survival rates of the groups in our clinical trial. Although there are former studies on this subject in the literature, it is seen that more studies are still being published. When the literature is examined on this subject, we think that the molecular differences may be the result of the heterogeneous groups in the studies. In addition, although the parameters examined in patients with early-stage tumors differ, it may be possible that this difference will disappear with advanced-stage tumors, as in our study group. New studies examining early-stage and late-stage tumors separately can be planned on this subject.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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