RESEARCH ARTICLE
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Prognostic Importance of Ki-67 Expression in Early-Stage Glottic Larynx Cancer Treated with Radiotherapy

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ABSTRACT

Objective: The prognostic importance of Ki-67 expression in early-stage glottic larynx cancer is controversial. In this study, we aimed to evaluate the prognostic importance of Ki-67 expression in early-stage glottic cancer treated with curative radiotherapy at a single centre.
Material and Method: Between 2010 and 2021, patients with T1a/bN0 stage glottic larynx cancer treated with curative radiotherapy were identified. The cases included in this study were re-evaluated by a single pathologist retrospectively. According to the ROC curve, the optimal cut-off value was 35% for Ki-67. Above these cut-off points were accepted as high expression levels (Ki-67-high), while below these points were accepted as low expression levels (Ki-67-low).
Results: A total of 49 patients with early-stage glottic larynx cancer treated with definitive radiotherapy were included. The median follow-up time was 35.74 (range; 7.03-161.65) months. The median OS was 95.4 (range; 62.1-128.8) months. According to univariate analysis, tumour grade (p=0.006) and Ki-67 expression level (low vs high; p=0.039) were the prognostic factors that predict OS. The median DFS was 42.1 (range; 32.8-51.3) months. According to univariate analyses, tumour grade (p=0.008) and Ki-67 expression level (low vs high; p=0.007) were the prognostic factors that predict DFS.
Conclusion: Higher Ki-67 expression was associated with lower OS and DFS. Ki-67 expression level can be used as a biomarker to determine treatment choice in these patients. Additional prospective studies with more patients are needed to confirm our results.
Keywords: Larynx Cancer, Ki-67, survival outcomes, radiotherapy

INTRODUCTION

Larynx cancer is one of the most common cancer types in head and neck malignancies (HNCs), and about 60% of cases originate from the glottis (1,2). The patients are often detected at an early stage, due to the tumour-associated hoarseness. Early detection provides an opportunity for organ preservation and cure (3). Radiotherapy, open partial laryngectomy, and transoral laser microsurgery are the current treatment options for early-stage glottic larynx cancer (3,4). Although the number of large, randomised studies is limited, the oncologic and quality-of-life outcomes of studies comparing both treatment modalities are similar (4). Institutional experience and the patient's preference often determine the treatment type. The 5-year local control rate varies between 80-95% for radiotherapy (3). Despite high control rates, salvage surgery in radioresistant tumours is usually performed as a total laryngectomy (1). Knowing the proliferation pattern of tumour cells before deciding on the treatment modality may guide the prediction of radio resistance and the choice of treatment.

In clinical practice, immunohistochemical determination of the Ki-67 proliferation index is usually used to evaluate the proliferation pattern of tumours (5). The prognostic importance of the Ki-67 proliferation index has been investigated in many types of cancer and also in HNCs (6). The studies showed that patients with a higher expression of Ki-67 had poorer outcomes. Additionally, a high Ki-67 expression is associated with a higher rate of lymph node metastasis (6) But, the prognostic importance of Ki-67 expression in early-stage glottic

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larynx cancer is controversial because of the heterogenous design of the studies that included patients with different stages of disease.

In the current study, we aimed to evaluate the prognostic importance of Ki-67 expression in early-stage glottic cancer treated with curative radiotherapy at a single centre.

MATERIALS AND METHODS

Patient population

Ethical approval of the study was obtained from the ethics committee of our university. The stage of disease was clinically determined according to the 8th edition of the TNM classification established by the American Joint Committee on Cancer (AJCC). The tumour is limited to the one vocal cord with normal mobility staged as T1a (may involve anterior or posterior commissure) and the tumour involves both vocal cords with normal mobility staged as T1b (may involve anterior or posterior commissure) (7). Between 2010 and 2021, the data of patients with T1a/bN0 stage glottic larynx cancer treated with curative radiotherapy were collected. Among them, whose histopathological identification was carried out at our university were included in this study. 49 patients' data from the hospital records, consisting of age, sex, Karnosky Performance Status, TNM status, sites of disease, grade of tumour, and treatment details, were evaluated retrospectively. All patients were newly diagnosed, biopsy-proven T1a/b stage squamous cell carcinoma of the glottis. The exclusion criteria were age < 18 years, Karnofsky Performance Status (KPS) < 70, history of another type of cancer within the last 5 years, previous head and neck radiotherapy, and follow-up time < 6 months.

Table 1: Patients, disease and treatment characteristics

Variables	No. of patients (Total:49)	%
Age (years)		
Median (range)	68 (45-83)	
≥68	27	55
<68	22	45
Sex		
Male	46	94
Female	3	6
Clinical stage*		
Stage T1a	38	78
Stage T1b	11	22
Anterior commissure involvement		
No		
Yes	34	70
Tumour Grade	15	30
Grade I		
Grade II	20	41
Grade III	17	35
Ki-67 expression level	12	24
Low-Ki-67		
High Ki-67	18	37
RT dose	31	63
6600 cGy		
6800 cGy	24	49
7000 cGy	8	16
	17	35

RT: Radiotherapy. * According to version of the American Joint Committee on Cancer (AJCC) 8th edition, 2017.

Treatment

All patients were examined by a multidisciplinary head and neck tumour board. The treatment decisions were routinely decided by board members based on functional and individual patient-related factors.

Radiotherapy

Radiotherapy was performed using a three-dimensional conformal technique with a linear accelerator. Typically, radiotherapy was applied with a pair of wedged lateral opposed fields using 6MV photon energy. The whole larynx was included in the target volume. Eclipse Treatment Planning System version 8.9.08 (Varian, Palo, Alto, CA) was used in treatment planning. The total radiation dose was 66-70 Gy/5 fraction per week according to clinician preference.

Follow-up

All patients were evaluated throughout the radiotherapy at least once a week. After the completion of radiotherapy, the patients were examined every 3 months for the first two years, every six months for the third year, and once a year thereafter. A historical, physical, and endoscopic examination was done at each follow-up visit. Additional imaging studies were done as needed to assess locoregional or distant failure.

Immunohistochemical determination

The cases diagnosed with early-stage glottic larynx cancer and included in this study were re-evaluated by a single pathologist (F.S.) retrospectively, according to the guideline recommendations of the American Society of Clinical Oncology/ College of American Pathologists (ASCO/CAP protocols) (8) Histological type and grading were redone from H&E-stained slides. The cases were histologically classified as keratinized and non-keratinized squamous cell carcinoma. The cases were graded as well differentiated (Grade-1), moderately differentiated (Grade-2), and poorly differentiated (Grade-3). 4 micron thick sections were taken from paraffin blocks of the cases on positively charged slides for immunohistochemical Ki-67 staining. Slides cut for the immunohistochemical study were incubated for 30 minutes in EDTA buffer at 97 degrees. Then, the immunohistochemical Ki-67 (DAKO-Clone-MIB-1) staining protocol was applied in the DAKO OMNIS machine. The stained slides were evaluated under the light microscope (Olympus BX 46) by the same pathologist. Ki-67; nuclearly positively stained cells were given a percentage as a hot spot.

Statistical analysis

Statistical Package for Social Sciences for Windows version 20 (SPSS. Inc. Chicago. IL) was used for all statistical analyses. Descriptive statistics were performed to evaluate the patient, disease, and treatment characteristics. The overall survival (OS)

Table 2: Univariate and multivariate Cox proportional hazard regression analysis related to OS

Univariate analysis Multivariate analysis Variables HR 95% CI p-value HR 95% CI p-value		
Tumour Grade		
Grade I	1 **0.05***	1
Grade II	1.96 0.43-8.82 0.37	1.01 0.20-5.16 0.98
Grade III	23.26 1.78-302.78 **0.01***	10.98 0.81-147.56 0.07
Ki-67 expression level		
Low Ki-67	1	1
High Ki-67	7.49 1.85-65.75 **0.02***	6.79 0.64-71.25 0.1

*Statistically significant

was calculated as the time from the pathological diagnosis to the date of death or last follow-up. The disease-free survival (DFS) was calculated as the time from the pathological diagnosis to the date of documented locoregional or distant recurrence/progression or the date of death or last follow-up. The local recurrence-free survival (LRFS) was calculated as the time from the date of pathological diagnosis and the first event of local recurrence. Kaplan–Meier analysis was carried out to measure the OS, DFS, and LRFS. The two-sided long-rank test was performed to make a comparison between the survival curves of subgroups. ROC (Receiver Operating Characteristics) curve analysis was performed to measure the ability of the ki-67 value to predict locoregional or distal recurrence/progression or death. The possible associations between Ki-67 and survival outcomes were measured by Cox regression analysis. If the two-sided p-value < 0.05, the outcome was mentioned as statistically significant.

RESULTS

Patients, disease, and treatment characteristics

A total of 49 patients with early-stage glottic larynx cancer treated with definitive radiotherapy between 2011 and 2021 were included in the current study. The median follow-up time was 35.74 (range; 7.03-161.65) months. The patient, disease, and treatment characteristics are summarized in Table 1.

Ki-67 evaluation

The median Ki-67 level was 40% (range: 10 to 90%; mean: 44.5%). According to the ROC curve, the optimal cut-off value was 35% for Ki-67. Above these cut-off points were accepted as high expression levels (Ki-67-high), while below these points were accepted as low expression levels (Ki-67-low).

Ki-67 expression and survival outcomes

During the follow up, local failure was observed in 9 of 49 patients (18%). Salvage surgery with total laryngectomy was performed in 8 out of 9 patients with local failure (%89). 37 patients were alive at the time of statistical analysis, and there was no disease either clinically or radiologically. Distant metastases were not seen in any of the patients.

The median OS was 95.4 (range; 62.1-128.8) months. The 2- and 5-year OS were 91% and 76%, respectively. Tumour grade (p=0.006) and Ki-67 expression level (high vs low; p=0.039) were the prognostic factors that predict OS, according to Kaplan Meier analysis (Figure 1a, 1b). The 2- and 5-year OS were 93% and 93% in the low expression group whereas 85% and 48% in the high expression group. To further evaluate these survival differences, we used a multivariate Cox regression analysis that controlled for other known prognostic factors. However, we did not show any association between prognostic factors

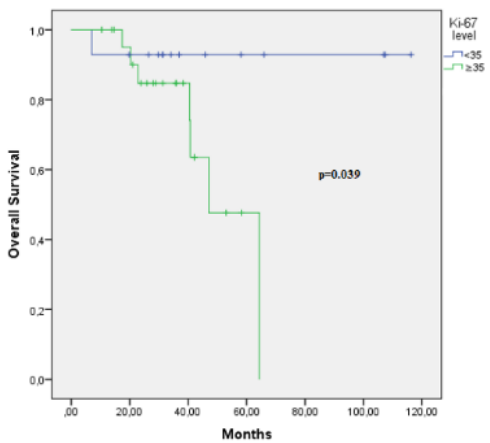

Figure 1a: Overall survival difference between Ki-67 level < 35% vs ≥ 35%.

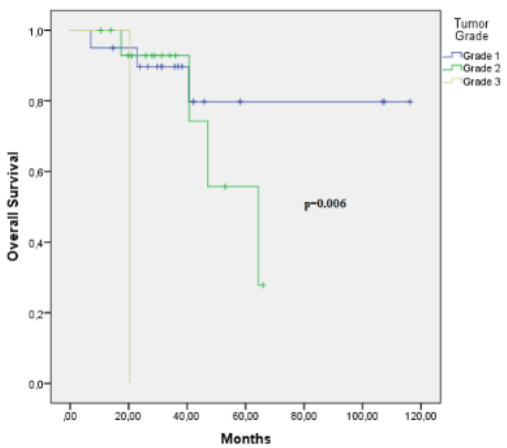

Figure 1b: Overall survival difference between grade1 vs grade2 vs grade3 tumours.

Table 3: Univariate and multivariate Cox proportional hazard regression analysis related to DFS

Univariate analysis Multivariate analysis Variables HR 95% CI p-value HR 95% CI p-value		
Tumour Grade		
Grade I	1 0.57	1 0.83
Grade II	0.91 0.28- 2.88 0.87	1.38 0.28-6.84 0.68
Grade III	3.24 1.06- 9.84 **0.038***	1.49 0.39-5.67 0.55
Ki-67 expression level		
Low-Ki-67	1	1
High Ki-67	6.60 1.41-30.86 **0.016***	6.69 1.04-32.36 **0.050***

*Statistically significant

according to multivariate Cox regression analysis. The median DFS was 64.3 (range; 44.8-83.8) months. The 2- and 5-year DFS were 89% and 56%, respectively. Ki-67 expression level (high vs low; p=0.007) and tumour grade (p=0.04) were the prognostic factors that predict DFS, according to Kaplan Meier analyses (Figure 2a, 2b). The 2- and 5-year DFS was 93% and 74% in the low expression group whereas 81% and 21% in the high expression group. According to multivariate Cox regression analysis, only the Ki-67 expression level was the prognostic factor that predicted DFS (p=0.05). The details of univariate and multivariate Cox proportional hazard regression analysis were summarized in Table 2 and Table 3.

According to Kaplan Meier analysis, both OS and DFS outcomes were shorter in patients with anterior commissure involvement when compared with no anterior commissure involvement, but the results did not reach statistical significance. The fact remains that local recurrence was found to be significantly higher in patients with anterior commissure extension after curative radiotherapy (p=0.01). The median LRFS was 116.2 (range; 62.1-190.3) months. The 2- and 5-year LRFS were 93% and 64%, respectively. Tumour grade (p=0.006) and Ki-67 expression level (high vs low; p=0.015) and anterior commissure involvement (no vs yes; p=0.019, Figure 3) were the prognostic factors that predict LRFS, according to Kaplan Meier analysis. To further evaluate these survival differences, we used a multivariate Cox regression analysis that also controlled for other known prognostic factors. We did not show any association between prognostic factors according to multivariate Cox regression analysis.

DISCUSSION

While investigating the prognostic effect of the Ki-67 proliferation index in HNCs, differences in tumour location, tumour stages, and treatment types make it difficult to clearly understand the prognostic impact of Ki-67 on survival (9,10). Even patients with the same stages and clinical features have differences in survival times. We aimed to overcome this problem by evaluating the prognostic significance of Ki-67 expression in only T1N0 stage glottic laryngeal cancer treated with radiotherapy. Despite very conflicted results that have been presented in the literature with the prognostic impact of Ki-67 expression in laryngeal cancer patients, overexpression of Ki-67 was found as an indicator of poor prognostic factors that related to survival outcomes according to our study results.

Some of the studies investigating the effect of Ki-67 expression in laryngeal cancer showed that Ki-67 expression is higher in poorly differentiated tumours and associated with more advanced TNM stage, higher lymph node metastases, and tumour recurrence. Additionally, overexpression of Ki-67 correlated with shorter DFS and treatment failure (9-11).

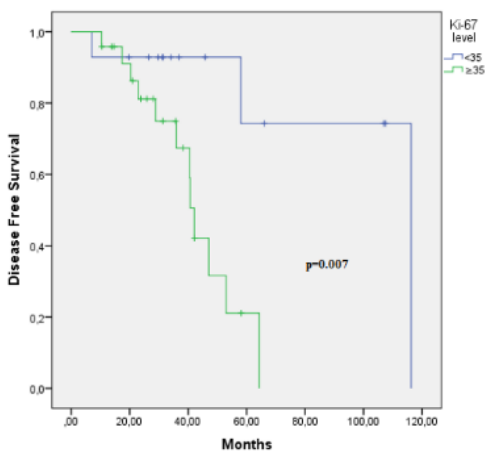

Figure 2a: Disease free survival difference between Ki-67 level < 35% vs ≥ 35%.

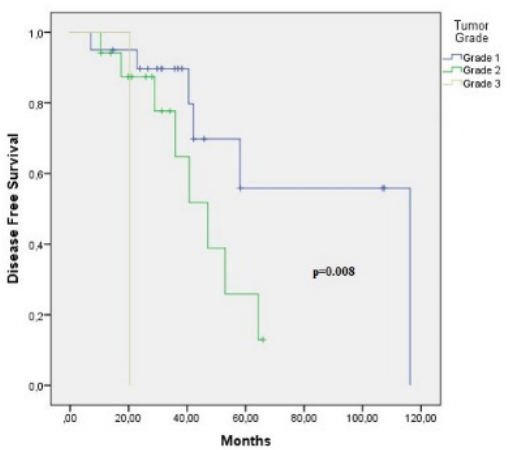

Figure 2b: Disease-free survival difference between grade1 vs grade2 vs grade3 tumours.

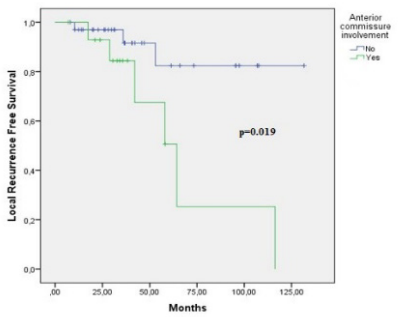

Figure 3: Local recurrence-free survival difference between with or without anterior commissure involvement.

Nevertheless, contradictory results have overall been published regarding radiotherapy. Kropveld et al. investigated the effect of Ki-67 expression on response to therapy in T2 glottic laryngeal cancer patients treated with radiotherapy and they found that tumours with higher Ki-67 expression respond significantly better to radiotherapy (12). Similarly, Ahmed et al. analysed the prognostic significance of Ki-67 expression in 24 glottic cancer involving the anterior commissure treated with radiotherapy and the authors reported that Ki-67 overexpression is a predictive marker for radiosensitivity (13).

Similar to our study results, Sakata et al. demonstrated that patients with T1 glottic cancer have a better local control rate when the Ki-67 index is less than 50% (14). In accordance with this study's results, Nichols et al. evaluated the predictive role of Ki-67 index in early-stage laryngeal cancer treated with radiotherapy and they found that overexpression of Ki-67 was significantly associated with tumour recurrence and predictor for lower DFS. They also concluded that Ki-67 expression may be a guide in identifying a group with an increased risk of local recurrence after radiotherapy and can potentially guide more effective personalized treatments in patients with early-stage glottic laryngeal cancers (15). Lavertu et al. evaluated the prognostic effect of Ki-67 expression in advanced-stage laryngeal cancer patients treated with concomitant chemo-radiotherapy. According to multivariate analysis, Ki-67 positivity was significantly associated with poor OS, and Ki-67 negativity was significantly associated with higher OS and organ preservation. Although the authors concluded that it was not appropriate to modify the treatment based on their results, they recommended closer follow-up in patients with Ki-67 positivity to detect possible recurrence earlier (16).

The other prognostic factor that predicts survival outcomes is anterior commissure involvement. Despite the suggestions that anterior commissure involvement predict a worse prognosis, it is not included in the staging system (17). We also evaluated the prognostic impact of anterior commissure invasion and we found that patients with anterior commissure involvement had a statistically significant shorter LRFS when compared with no anterior commissure involvement. The mean LRFS was 66 months for patients with anterior commissure involvement whereas 115 months for patients without anterior commissure involvement. Additionally, local recurrence was found to be significantly higher in patients with anterior commissure extension after curative radiotherapy (p=0.01). Anterior commissure invasion was detected in 15 of 49 patients (%30) in the current study. Compared to the literature, our relatively high rates of local recurrence may also be associated with anterior commissure involvement in one-third of our patients.

The limitations of our research were its retrospective design and relatively limited number of patients. Due to the retrospective design, analysis of parameters that will affect local recurrence and survival, such as the continuation of smoking and alcohol usage after treatment, could not be performed. We think that it will contribute to the literature in terms of the fact that it was performed on a homogeneous patient group treated in a single centre with a similar treatment protocol and that Ki-67 expression analysis was re-evaluated by a single pathologist. Additionally, our study is important in terms of guiding prospective studies on this subject.

CONCLUSION

In conclusion, according to our study, higher Ki-67 expression was related to lower OS and DFS in early-stage glottic larynx cancer treated with radiotherapy. Ki-67 expression level can be a guide for determining treatment selection in these patients. Additional prospective research with more patients is needed to validate our results.

Ethics Committee Approval: This study was approved by the Ethics Committee of Necmettin Erbakan University (Date: 27.10.2022, No: 161).

Informed Consent: Written informed consent was obtained.

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- G.K.; Data Acquisition- G.K., S.F., B.B.Y., M.A., F.C.E.; Data Analysis/Interpretation- G.K.; Drafting Manuscript- G.K., S.F.; Critical Revision of Manuscript- G.K., S.F.; Final Approval and Accountability- G.K., S.F., B.B.Y., M.A., F.C.E.

Conflict of Interest: The authors have no conflict of interest to declare.

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