Predictive value of ALDH1 and CD44 positivity for radiotherapy response and prognosis in early-stage glottic laryngeal squamous cell carcinoma

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Abstract

Objective: To evaluate of the predictive value of CD44 and ALDH1 expression for prognosis and radiotherapy(RT) response in patients with early-stage laryngeal cancer receiving RT. Materials and Methods: Forty-four patients with early-stage laryngeal cancer were included in the study. Based on retrospective chart review, the patients were divided into those with local recurrence and those without recurrence after RT. Correlation between RT response and pre-treatment immunohistochemical ALDH1 and CD44 staining was evaluated. In addition, survival times were compared between groups. Results: The mean age was found 59.8±9.0 years and 41 were male. There were 20 patients in the non-recurrent group. Immunohistochemical positivity for ALDH1 was found to be significant risk factor for RT failure (p=0.0001), whereas CD44 positivity (p=0.114) were not significant. Disease-free survival was shorter in cases with positive ALDH1 and CD44 staining (p=0.0001). Conclusion: ALDH1 positivity was identified as a significant predictor of DFS and RT sensitivity.

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Abstract

Background: Several prognostic factors can be used for the evaluation of laryngeal cancer. The aim of this study to evaluate of the predictive value of CD44 and ALDH1 expression for prognosis and radiotherapy(RT) response in patients with early-stage laryngeal cancer receiving RT.

Methods: Correlation between RT response and pre-treatment immunohistochemical ALDH1 and CD44 staining was evaluated. In addition, survival times were compared between groups.

Results: The mean age was found 59.8±9.0 years and 41 were male. There were 20 patients in the non-recurrent group. Immunohistochemical positivity for ALDH1 was found to be significant risk factor for RT failure (p=0.0001), whereas CD44 positivity (p=0.114) were not significant. Disease-free survival was shorter in cases with positive ALDH1 and CD44 staining (p=0.0001).

Conclusion: ALDH1 positivity was identified as a significant predictor of DFS and RT sensitivity.

Keywords: Laryngeal squamous cell carcinoma, ALDH1, CD44, radiotherapy

What's already known about this topic?

- At present, there are various treatment modalities for treating early glottic cancer; namely, RT or partial laryngectomy techniques.
- Recent studies have shown similar local control between RT and surgery.
- Several prognostic factors can be used for the evaluation of laryngeal cancer.
- Some markers such as CD44, CD133, ALDH1, and ABCG2 are used to determine of predictivity of prognosis.

What does this article add?

- ALDH1 positivity was associated with disease-related survival and RT sensitivity.
- CD44 positivity did not differ between patients with and without recurrence after RT.

Introduction

Although new treatment protocols for head and neck cancer have been developed in recent years, head and neck cancer is still the sixth most common cancer worldwide, mainly due to its association with human papillomavirus (HPV) and tobacco and alcohol usage (1) . The most common histopathological type is squamous cell carcinoma (SCC) and the oral cavity, larynx, and pharynx are most frequently involved (2) .

Laryngeal SCC is the most common head and neck cancer, affecting an estimated 100,000 people per year (3). For patients who present with advanced disease, the mainstay of treatment is total laryngectomy with or without adjuvant therapy (4) . However, the survival rate is low. Among laryngeal cancers, glottic carcinomas are the most common subgroup, with the glottis being involved approximately 3 times more often than the supraglottic larynx. Glottic cancers are usually diagnosed in the early stage due to symptoms of hoarseness.

In early glottic cancer, lymph node metastasis is rarely seen, with an incidence of clinically positive lymph nodes of nearly zero for stage T1 and $< 2\%$ for stage T2 disease, and a complete cure can often be achieved by radiotherapy (RT) or surgery (5-7). Therefore, the goal is to achieve the best local control leading to a complete cure and optimal functional results. At present, there are various treatment modalities for treating

early glottic cancer; namely, RT or partial laryngectomy techniques. Although surgery has been used for decades, its use has greatly decreased in recent years because of declining functional results and advances in RT (8). The optimal treatment for early glottic cancer has remained an issue of debate, primarily due to a lack of evidence from large prospective randomized trials (9). Recent studies have shown similar local control between RT and surgery. Mendenhall et al. reported local control rates ranging from approximately 80% to 94% for T1 tumors and 70% to 85% for T2 tumors with both modalities (10,11).

Several prognostic factors can be used for the evaluation of laryngeal cancer. Microscopic grade is an independent prognostic factor and correlates with clinical stage (12). Recurrence is related to aneuploidy (13). The presence of S100-positive Langerhans cells around the tumor is called host reaction and has been associated with favorable prognosis (14).

The most accepted prognostic factors are TNM classification. However, the TNM system cannot distinguish aggressive tumors from nonaggressive tumors of the same size. Identifying one or more biomarkers to predict the biological behavior of head and neck squamous cell carcinomas (HNSCCs) would be beneficial. Recently, a small population of cancer cells referred to as cancer stem cells (CSCs) was found to be responsible for tumor initiation, relapse, and resistance to chemotherapy or RT; therefore, eradicating CSCs is considered critical in cancer therapy (15,16). The CSC hypothesis has also been proposed for HNSCCs; some cell surface markers have been reported as CSC markers in HNSCC, such as CD44, CD133, ALDH1, and ABCG2 (17-19), and high expression of these markers is usually regarded as an indicator of poor prognosis. Among them, CD44 is the most reported CSC marker in HNSCC (20-22).

In this study, we aimed to evaluate of the predictive value of CD44 and ALDH1 expression for prognosis and treatment response in patients with early-stage laryngeal cancer receiving RT.

Materials and Methods

Forty-five patients diagnosed as having early-stage laryngeal cancer and treated with RT in the otorhinolaryngology department of XXX University Faculty of Medicine between 2002 and 2016 were included in the study. Patients were treated with curative radiotherapy with a linear accelerator with a peak energy of 6 MV. All patients were treated Patients were treated with 225 cGy /28 days in the same radiotherapy center. The demographic and clinical data, pathology reports, prognostic parameters, and survival rates of the patients were retrieved retrospectively from their medical records. Among patients with early-stage patients with recurrent and Cure with the same sampling were studied. Patients with anterior commissure involvement were excluded from the study. The patients were divided into two groups, those who showed complete response with RT (non-recurrent group) and those who developed local recurrence despite RT (recurrent group). The correlation between treatment response and immunohistochemical staining for ALDH1 and CD44 at time of diagnosis was evaluated. Survival rates were also compared between the groups.

Histopathological analysis

For each patient, slides stained with hematoxylin-eosin were reviewed by the same pathologist, tumor tissues were selected, and ALDH1 and CD44 immunohistochemistry was performed on these tissues. For immunohistochemical staining, 4-micron thick sections were obtained from paraffin-embedded blocks in positively charged glasses and then deparaffinized. Rehydration, blockade with hydrogen peroxide, and 20 minutes of antigen retrieval was performed with sodium citrate buffer in a microwave. Samples were incubated overnight at 2–8°C with anti-ALDH1 rabbit monoclonal antibody (ab9883, Abcam) (1:500) (5 µg/ml) and CD 44 antibody (1:50) (Santa Cruz). After incubation with antibody, samples were stained with DAB chromogen. Finally, all samples were stained with Mayer hematoxylin and washed with distilled water and PBS.

For immunohistochemical analysis, a semiquantitative evaluation was performed. The intensity and extent of staining were scored for ALDH1. Density was scored as 0: no staining; 1: weak staining; 2: moderate staining; 3: strong staining. Extent of staining was evaluated based on the percentage of positive cells. A total score was obtained by adding the density and extent scores. The evaluation of CD44 staining was based on membranous staining and was performed using the same scoring method.

Statistical analyses

Chi-square test or Fisher's exact test were used to compare the groups. The Kaplan–Meier method and Cox proportional regression model were used to estimate the mean/median survival rates, failure rates, and hazard ratios (HRs). Log-rank test was used to compare the survival distributions between groups. The prognostic ability of ALDH1 and CD44 were evaluated for DFS in both univariate and multivariable analyses. DFS was defined as the time from diagnosis to any documented clinical progression, relapse, or death from any cause. The results were reported as mean±SD, median, number (n) and percent (%). A p value < 0.05 was considered significant. The analyses were performed using the IBM SPSS Statistics version 22.0 (IBM Corp., Armonk, NY).

Results

Forty-four patients with early-stage laryngeal cancer and treated with RT were retrospectively evaluated. Their mean age was 59.8±9.0 (43–81) and 41 were men. Before treatment, all patients were confirmed as having early-stage disease according to the findings of indirect laryngoscopic and radiologic evaluation and were reported as SCC according to pre-treatment biopsy. Thirty-four patients had T1 glottic tumor and 11 had T2 glottic tumors. There were not anterior commissure involvement seen at all patients. All of the patients were diagnosed as early-stage glottic carcinoma according to the classification system of AJCC 2017 (23).

There were 20 patients (all men) in the non-recurrent group and 24 patients (22 men and 3 women) in the recurrent group. Median follow-up time was 48 months (22–88 months). Treatment failure was observed in 24 cases. Table 1 shows the demographic and clinical features of the patients.

Table 2 shows demographic and clinical features of the patients according to treatment response. While positive ALDH1 staining (p=0.0001) was found to be significant risk factor for treatment failure, positive CD44 staining (p=0.114) and age group (p=0.287) were not significant factors.

Means and medians for disease-free survival (DFS) time are shown in Table 3. The mean OS was 60.7 months (median: 62.0 months). Cumulative proportion of surviving patients was 81% at 12 months (1 year), 56% at 24 months (2 years), and 42% at 36 months (3 years). Figure 1 shows total DFS times. DFS was shorter in cases with positive ALDH1 staining and CD44 staining (p=0.0001); Figure 2A shows DFS times according to ALDH1 positivity and Figure 2B shows DFS times according to CD44 positivity.

Table 4 shows results of the Cox regression analyses. Two Cox regression models were created using significant parameters detected in univariate survival analysis: age, ALDH1 positivity, CD44 positivity. According to Cox regression model, only the ALDH1 positivity was found to be a significant independent factor for DFS, with positivity associated significantly increasing the poor prognosis HR:16.4 (95% CI: 3.6-73.5, p=0.0001).

Discusion

Recently, a small population of cancer cells referred to as CSCs has been implicated in tumor initiation, relapse, and resistance to chemotherapy or RT; as a result, eradication of CSCs is considered essential in cancer therapy (15,16). The CSC hypothesis has also been coined for HNSCC in the head and neck; some cell surface markers have been reported as CSC markers in HNSCC cancers, such as CD44, CD133, ALDH1 and ABCG2 (17-19), and high expression of these markers is usually considered an indicator of poor prognosis. Among them, CD44 is the most reported CSC marker in HNSCC (20-22). Chen et al. reported that ALDH1-positive cells are phenotypic and functional precursors of CSCs. These cells develop from ALDH1 cell lines and have more proliferative activity (24). In the same study, it was shown that ALDH1-positive cells had similar genetic structure to mesenchymal stem cells. Guided by these studies, ALDH1 expression is thought to be a good biomarker for CSCs in head and neck cancers (24,25).

A study by Chen et al. showed that colony formation was stimulated with 0–10 Gy radiation in isolated ALDH1-positive cells. The study also compared CD44-positive, CD24-negative cells that were positive and negative for ALDH1. A total of 226 HNSCC patients who were positive for ALDH1 were evaluated, and

ALDH1 levels were found to be associated with advanced-stage disease and undifferentiated tumors. In addition, it was determined that patients who were positive for ALDH1 had poorer survival with oncological treatment (26). In an experimental study, authors reported that a small portion of the population of CD 44 possitive cancer cells, which typically comprise < 10 % of the cells in a head and neck squamous cell carcinoma tumor, but not the CD44 negative cancer cells, gave rise to new tumor in vivo. Immunohistochemistry showed that the CD44 possitive cancer cells have a primitive cellular structure and costain with the basal cell marker Cytokeratin 5/14, whereas the CD44 negative cancer cells resemble differentiated squamous epithelium and express the differentiation marker Involucrin. The tumors that arose from purified CD44 possitive cells reproduced the original tumor heterogeneity and could be serially passaged, thus demonstrating the two defining properties of stem cells: ability to self-renew and to differentiate (27).

In our study, the prevalence of ALDH1 positivity was significantly higher among patients with tumor recurrence due to RT failure. Al-Assar et al. reported that several biomarkers such as CD44, CD24, CD133, and epithelial-specific antigen may be related to radiosensitivity. In this study, it was found that CSCs, except those that were CD24-negative, did not have a radioresistant phenotype (28). In a meta-analysis, ALDH1 expression was reported to be associated with low overall and disease-related survival (29). In contrast, Lopez-Gonzales reported that patients with stage 1-2 lung cancer and ALDH1 nuclear expression showed good survival (30). This seems completely opposite of our study, in which ALDH1 positivity was significantly more common among patients with failed RT while CD44 positivity was not significantly different between the two groups. ALDH1 positivity was found to be an independent risk factor for DFS and significantly associated with poor prognosis.

This study has limitations such as the small sample size, and not specifying the smoking status and comorbidities of the patients. Because patients with anterior commissure involvement excluded from the study, sample size is small.

Conclusion

In conclusion, the results of our study demonstrate that ALDH1 positivity was associated with disease-related survival and RT sensitivity. CD44 positivity did not differ between patients with and without recurrence after RT. In our study, sample size is small and needs to be done larger cohort studies for the powered the predictive effects of these markers. For ALDH1 positivity to be used as a biomarker when making treatment decisions, it is necessary to conduct studies with a larger patient series and determine its correlation with other biomarkers.

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Conflicts of Interest

The authors report no conflicts of interest.

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Ethics

Ethics committee approval of the study was obtained from the ethics committee of the University of Çukurova (**approval number-March 3,2017;62/27).** The study was carried out in accordance with the principles of the Helsinki Declaration.

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Figure legends

Figure 1: Total DFS curve.

Figure 2: DFS curves according to ALDH1 positivity (A) and CD44 positivity (B).

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