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Low Expression of pRB Predicts Disease Relapse in Early Glottic Cancer Treated With Transoral Laser Microsurgery

Li-Ang Lee, MD, MSc, FICS; Tuan-Jen Fang, MD, FICS; Hsueh-Yu Li, MD, FACS; Chung-Guei Huang, PhD; Tse-Ching Chen, MD, PhD; Chun-Ta Liao, MD; Chung-Jan Kang, MD; Kai-Ping Chang, MD, PhD; Tzu-Chen Yen, MD, PhD

Objectives/Hypothesis: To elucidate the associations among the immunohistochemical expression of tumor markers, clinicopathological variables, and disease-free survival (DFS) in patients with early-stage glottic squamous cell carcinoma (SCC) who underwent transoral laser microsurgery (TLM) as the primary treatment.

Study Design: Retrospective chart review.

Methods: The records of consecutive patients with Tis–T2N0 glottic SCC who underwent TLM between August 1, 2012 and October 31, 2015 were reviewed. Expression of Bcl-2, pRB, p16INK4A, p53, c-Myc, E-cadherin, and EGFR was examined using tissue microarrays containing tumor specimens through immunohistochemistry. Three-year DFS rates were calculated.

Results: A total of 65 consecutive patients were identified, of which 28 were excluded due to insufficient tissue (n = 22) and low biomarker quality (n = 6). Therefore, 37 patients with complete records were included. The included patients were significantly older and had a more advanced type of cordectomy than did the excluded patients (P = .015 and .009, respectively). According to the findings of univariate analysis, age, betel quid chewing, type of cordectomy, BCL-2 expression, and pRB expression significantly predicted 3-year DFS. According to the findings of multivariate analysis, age (adjusted hazard ratio: 0.94, 95% CI: 0.88-1.00), betel quid chewing (adjusted hazard ratio: 5.07, 95% CI: 1.32-19.44), and pRB expression (adjusted hazard ratio: 0.02, 95% CI: 0.00-0.28) were independent predictors of 3-year DFS.

Conclusions: Low pRB expression is a potential biomarker for predicting disease relapse after primary TLM for early-stage glottic SCC and may help to identify high-risk patients who can subsequently undergo intensive management.

Level of Evidence: 4

Laryngoscope, 129:E220–E226, 2019

INTRODUCTION

Laryngeal cancer is one of the most common cancers worldwide. The excellent functional and oncological outcomes of the treatment of early-stage laryngeal cancer enable patients and clinicians to select radiotherapy (RT) or transoral laser microsurgery (TLM) according to their own preference and the availability of these treatment modalities.1–3 TLM is a cost-effective treatment modality for managing early-stage laryngeal cancer.1,4 In our recent study,5 we found that patients who had received an early diagnosis of laryngeal cancer experienced the worsening of voice and quality of life immediately after undergoing TLM; however, these were gradually restored.

Although both TLM and RT aim to treat early-stage glottic squamous cell carcinoma (SCC) successfully while causing few complications and preserving functions to a satisfactory extent, their 5-year disease relapse (mainly local recurrence [LR]) rates remain high.2,3 For example, there was a trend toward improved survival and increased LR of invasive cancer in patients with glottic tumor in situ (Tis) treated by surgery alone compared to RT or surgery with RT in more recent decades.6 Two separate meta-analyses of the oncological outcomes of TLM and RT for T12 and T23 glottic SCC have suggested that these modalities have equivalent local control rates; however, TLM results in significantly superior overall survival compared with RT.2

In cases of early-stage glottic SCC treated using TLM, increased stromal chronic inflammation was correlated with increased 1-year disease-free survival (DFS).7 Recently, two retrospective studies of patients with Tis–T3 glottic SCC treated using TLM indicated that several clinicopathological risk factors, such as age, margin
status, arytenoid cartilage invasion, positive surgical
tumor markers to develop more accurate predic-
tors of clinical outcomes.

Molecular markers representing
apoptosis regulators (such as Bel-2),
cell cycle regulators (such as p16INK4A),
multifunctional regul-
ators (such as c-Myc protein),
tumor suppressors (such as pRB and E-cadherin),
and the ErbB family of recep-
tors (such as EGFR and E-cadherin), and the ErbB family of recep-
tors (such as EGFR). These markers are known to express the markers of interest) were included as
positive controls (tissues known to express the markers of interest) were included as
appropriate. Tissue sections 4-μm thick were prepared for immu-
nohistochemical staining.

Staining was performed at room temperature using an
automated immunostainer (Bond-Max; Leica Microsystems
GmbH, Wetzlar, Germany), according to the manufacturer’s
instructions. The antibodies against Bel-2 (CAT # M088701;
Dako North America, Inc., Carpinteria, CA) (Fig. 1A), pRB (CAT
# NCL-RB-358; Leica Biosystems Ltd., Newcastle, United King-
dom) (Fig. 1B), p16INK4A (CAT # E6H4; Roche Diagnostics
GmbH, Heidelberg, Germany) (Fig. 1C), p53 (CAT # NCL-
P53-D07; Leica Biosystems Ltd.) (Fig. 1D), c-Myc (CAT # C22258;
Zeta Corp., Sienna Madre, CA) (Fig. 1E), E-cadherin (CAT # E-
CAD-L-CE; Leica Biosystems Ltd.) (Fig. 1F), and EGFR (CAT #
Z2037; Zeta Corp.) (Fig. 1G) proteins were used for IHC. To
evaluate optimal detection, antibodies were diluted at the following
ratios: 1:30 (p16INK4A), 1:50 (c-Myc), 1:100 (pRB, EGFR, and E-
cadherin), 1:200 (p53), and 1:500 (Bcl-2). Anti-EGFR and anti-E-
cadherin reactions were performed for 30 minutes, and the other
antibody reactions were performed for 20 minutes.

Patient Cohorts
A total of 65 consecutive patients who had received a new
diagnosis of early glottic SCC underwent endoscopic biopsy and
TLM at the Department of Otorhinolaryngology–Head and Neck
Surgery, Linkou-Chang Gung Memorial Hospital (a tertiary med-
ical center) in Taoyuan City, Taiwan, between August 1, 2012
and October 31, 2015. The inclusion criteria of this study were 1) age > 18 years and 2) a pathological diagnosis of Tis-T2N0 glottic
SCC. The exclusion criteria included 1) inadequate tumor
tissue for immunohistochemistry (IHC) examinations and 2) low
biomarker quality.

Procedures
All patients were extensively evaluated. Disease staging was
performed according to the seventh edition of the American Joint
Committee on Cancer Tumor Node Metastasis Staging System.
En bloc resection of the laryngeal tumor was performed by a lar-
yngologist (L.-A.L., T.-J.F., or H.-Y.L.) through TLM; tumor-free mar-
gin status was confirmed using frozen-section analysis during the
operation.5,7,12 In the patients who had positive primary excision margin(s) in the frozen-section analysis, additional extended
resections were performed until all secondary margins were deter-
mined to be negative for malignancy. The surgical technique of
TLM was described previously,12 and the types of cordecotomy
were recorded according to the European Laryngological Society
classification system.13,14 The management strategies for the
patients were reviewed by a multidisciplinary tumor board at the
hospital. Disease relapse, including LR, neck recurrence, and dis-
tant metastasis, was defined as positive biopsy, cytology, or imaging
after undergoing TLM with a negative posttreatment
screening. DFS was defined as the time from the definite treat-
ment to any disease relapse or the last known follow-up.

IHC Staining
Routine hematoxylin and eosin–stained slides prepared from
formalin-fixed, paraffin-embedded tissue blocks were histo-
logically reviewed for determining the lesion type and tumor
tissue adequacy. Laryngeal cancer foci were marked on the
corresponding hematoxylin and eosin–stained slides by a pathol-
gist (T.-C.C.). Two areas per case were determined for assembling
the recipient blocks to ensure suitable representation of the
selected cores. Tissue core biopsies (1.0 mm in diameter) were
harvested from the corresponding donor block and placed into a
tissue microarray (TMA) recipient block using a tissue processor
(Beecher Instruments, Sun Prairie, WI). Negative controls (exclu-
sion of the primary antibody) and positive controls (tissues
known to express the markers of interest) were included as
appropriate. Tissue sections 4-μm thick were prepared for immu-
nohistochemical staining.

This study investigated whether the immunohisto-
chemical expression of molecular markers predicted dis-
ease relapse in patients with early-stage (Tis–T2N0M0) glottic SCC who underwent solitary TLM as the primary treatment.

MATERIALS AND METHODS

Ethics Statement
The institutional review board of the Chang Gung Medical
Foundation approved this retrospective study (No. 201701338B0),
and the requirement for patients’ informed consent was waived.

Statistical Analysis
The main study endpoint was DFS. All of the patients
underwent follow-up examinations for a minimum of 6 months
after surgery or until death, and follow-up visits were continued
until December 2017. After applying the D’Agostino and Pearson
normality test, most variables were found to have a nonnormal distribution, and the descriptive statistics of these variables are presented as the median and range. Differences between groups were analyzed using the Mann-Whitney U test for continuous variables and the χ² test or Fisher exact test for categorical variables, as appropriate. Survival curves were plotted using the Kaplan-Meier method and compared using the log-rank test. Hazard ratios and their corresponding 95% confidence intervals (CIs) were calculated using Cox proportional hazard regression models. The effects of variables that were associated with the outcome and had a P value of ≤.20 after univariate analysis were assessed without discretizing continuous variables for reducing type I error° by using a backward, stepwise, multivariate Cox proportional hazard regression model. Spearman correlation coefficients were computed between selected predictors. All statistical analyses were conducted using SPSS software (version 24; IBM Corp., Armonk, NY) and Graph Pad Prism (version 7.00; Graph Pad Software Inc., San Diego, CA). A two-tailed P value of <.05 was considered statistically significant.

RESULTS

Of the 65 patients, 37 with complete records were included, and the remaining 28 were excluded due to

![Fig. 1. Representative examples of positive expression of the tumor markers examined in this study: (A) Bcl-2. (B) pRB. (C) p16INK4A. (D) p53. (E) c-Myc. (F) E-cadherin. (G) EGFR. Original magnification: 40x.](image)
inadequate tumor tissue (n = 22) or low biomarker quality (n = 6). Table I lists the characteristics of the included and excluded patients. The patients in the included group were significantly older than those in the excluded group (P = .015), and the included group had more advanced cordectomy compared with the excluded group (P = .009). Differences in other characteristics did not reach statistical significance.

The median follow-up duration of all of the patients was 41 months (range, 23–64 months). As of December 2017, 64 patients were alive, and one patient had died from a second primary cancer in the third year after diagnosis; 16 patients waited for further follow-up visits. Thirteen patients developed LR (median = 15 months; range, 4–30 months), none had neck recurrence, and none experienced distant metastasis. Thus, the 3-year DFS rate of the entire group was 80% (95% CI: 70%-90%). Twelve patients developed LR in the included group, and one patient developed LR in the excluded group. The 3-year DFS rate of the included group was significantly lower than that of the excluded group (67% [95% CI: 52%-83%] vs. 96% [95% CI: 89%-100%], P = .004).

The cellular expression of Bcl-2, p16INK4A, E-cadherin, and EGFR and the nuclear expression of pRB, p53, and c-Myc in terms of the PI and HS appeared heterogeneous in the included group (Table II). Figure 1 shows representative samples of the expression of the investigated tumor markers.

The results of univariate Cox proportional hazard regression models revealed that age, betel nut chewing, type of cordectomy, Bcl-2 expression, and pRB expression significantly predicted the 3-year DFS rate in the included group (Table III). Male sex, cigarette smoking, alcohol drinking, pathological T stage, and expression of p16INK4A, p53, c-Myc, E-cadherin, and EGFR were not significantly related to disease recurrence. Notably, the HSs of all the tumor markers did not significantly predict the 3-year DFS rate and were applied in further analyses.

Age, betel quid chewing, type of cordectomy, and expression of Bel-2, pRB, and p16INK4A were included in multivariate analyses in the included group. Male sex was not included in further analyses because of the presence of only one woman in the sample. Age was significantly and inversely associated with betel quid chewing (Table IV). Betel quid chewing was significantly and inversely associated with age and Bel-2 expression. The type of cordectomy was significantly and inversely correlated with pRB expression. Bel-2 expression was significantly and inversely associated with betel quid chewing and positively associated with p16INK4A.

After performing the multivariate analysis (Table V), age and pRB expression (nuclear PI) were identified as independent predictors of a lower 3-year DFS rate, whereas betel quid chewing was identified as an independent predictor of a higher 3-year DFS rate in the included group.

**DISCUSSION**

In this study, the expression of Bcl-2 and pRB was identified to predict the 3-year DFS rate in patients with Tis-T2 glottic SCC based on the findings of univariate analysis. However, the predictive value of Bcl-2 expression became less significant after adjustment with betel quid chewing. A combination of age, betel quid chewing, and pRB expression can help identify high-risk patients with Tis-T2 glottic SCC undergoing TLM who require intensive management.

pRB is a tumor suppressor protein that is dysfunctionally expressed in head and neck SCC; however, its role in disease relapse remains controversial. In head and neck SCC, G1/S transition regulators, such as cyclin D1

---

**TABLE I.**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Included, n = 37</th>
<th>Not Included, n = 28</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr, median (range)</td>
<td>66 (42–89)</td>
<td>54 (37–80)</td>
<td>.015</td>
</tr>
<tr>
<td>Male sex, no. (%)</td>
<td>36 (97)</td>
<td>23 (82)</td>
<td>.077</td>
</tr>
<tr>
<td>Cigarette smoking, no. (%)</td>
<td>34 (92)</td>
<td>23 (82)</td>
<td>.275</td>
</tr>
<tr>
<td>Alcohol drinking, no. (%)</td>
<td>29 (78)</td>
<td>17 (61)</td>
<td>.170</td>
</tr>
<tr>
<td>Betel quid chewing, no. (%)</td>
<td>15 (41)</td>
<td>8 (29)</td>
<td>.433</td>
</tr>
<tr>
<td>Pathologic T stage, no. (%)</td>
<td></td>
<td></td>
<td>.660</td>
</tr>
<tr>
<td>Tis</td>
<td>6 (16)</td>
<td>6 (21)</td>
<td></td>
</tr>
<tr>
<td>T1a</td>
<td>11 (30)</td>
<td>11 (39)</td>
<td></td>
</tr>
<tr>
<td>T1b</td>
<td>9 (24)</td>
<td>4 (14)</td>
<td></td>
</tr>
<tr>
<td>T2</td>
<td>11 (30)</td>
<td>7 (39)</td>
<td></td>
</tr>
<tr>
<td>Cordectomy type, no. (%)</td>
<td></td>
<td></td>
<td>.009</td>
</tr>
<tr>
<td>Type I</td>
<td>0 (0)</td>
<td>5 (18)</td>
<td></td>
</tr>
<tr>
<td>Type II</td>
<td>7 (19)</td>
<td>11 (39)</td>
<td></td>
</tr>
<tr>
<td>Type III</td>
<td>13 (35)</td>
<td>2 (7)</td>
<td></td>
</tr>
<tr>
<td>Type IV</td>
<td>3 (8)</td>
<td>3 (11)</td>
<td></td>
</tr>
<tr>
<td>Type V</td>
<td>8 (22)</td>
<td>4 (14)</td>
<td></td>
</tr>
<tr>
<td>Type VI</td>
<td>6 (16)</td>
<td>3 (11)</td>
<td></td>
</tr>
</tbody>
</table>
and p16<sup>NK4A</sup>, can be used as possible biomarkers of disease relapse. However, pRB expression was associated with disease recurrence and death in patients with oropharyngeal cancer.

Approximately one-tenth of patients with laryngeal SCC were found to have loss of heterozygosity for the RB locus (13q14) associated with pRB expression, and low pRB expression was related to LR. Mizokami et al. found that loss of pRB expression was associated with invasive tumor behaviors, such as a high T classification or histological grade, and predicted disease relapse. By contrast, Morshed et al. reported that high pRB expression was correlated with an advanced tumor stage and a low overall survival rate in laryngeal SCC. In the present study, we demonstrated that low pRB expression could significantly predict DFS and suggested that a combination of patient and molecular characteristics may be optimal in predicting therapeutic responses in laryngeal SCC.

Age appears to be a major predictor of DFS among patients with laryngeal SCC; however, it can affect the occurrence of disease relapse differently depending on the population and treatment modality. For example, age > 60 years was a risk factor for lower DFS in Italian patients with early-to-intermediate cancer treated using TLM. In patients with glottic SCC undergoing RT or TLM in Norway, age <70 years was an independent risk factor for disease relapse. We found that younger age was significantly associated with a lower DFS rate in Taiwanese patients with early-stage glottic SCC. Therefore, when evaluating the prognostic value of molecular markers, controlling for age is mandatory.

In an epidemiological study, betel quid chewing was identified to increase the risk of oral cavity SCC but reduce the risk of larynx SCC. However, individuals who both consumed alcohol and chewed betel quid experienced a stepwise increase in the cumulative risk of larynx SCC. In patients with head and neck SCC in Taiwan (a betel quid–prevalent region), microsatellite instability in the tumor-free surgical margins was associated with LR. These findings may support our observation that betel quid chewing increased the risk of disease relapse. To the best of our knowledge, this is the first study to consider betel quid chewing as a risk factor for DFS in laryngeal cancer. Thus, additional studies considering betel quid chewing are warranted to understand its underlying mechanism in early-stage glottic SCC.

Although an exploratory study failed to find any significant predictive value of the expression of Bcl-2 and p53 with respect to survival or relapse in laryngeal cancer treated with RT, we agree that protein profiling using TMAs and IHC is a feasible strategy for prognostic and predictive biomarker screening in laryngeal cancer. Molecular markers evaluated using IHC staining have recently been reported to potentially identify clinically relevant features associated with response to chemotherapy in patients with head and neck SCC and LR in

### Table II

<table>
<thead>
<tr>
<th>Tumor Markers</th>
<th>Location</th>
<th>Positive Index (%)</th>
<th>Histologic Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bcl-2 expression</td>
<td>Cell</td>
<td>86.7</td>
<td>26.5–100.0</td>
</tr>
<tr>
<td>pRB expression</td>
<td>Nucleus</td>
<td>0.54</td>
<td>0.09–0.84</td>
</tr>
<tr>
<td>p16&lt;sup&gt;NK4A&lt;/sup&gt; expression</td>
<td>Cell</td>
<td>47.6</td>
<td>1.8–99.3</td>
</tr>
<tr>
<td>p53 expression</td>
<td>Nucleus</td>
<td>0.14</td>
<td>0.01–0.67</td>
</tr>
<tr>
<td>c-Myc expression</td>
<td>Nucleus</td>
<td>0.42</td>
<td>0.05–0.71</td>
</tr>
<tr>
<td>E-cadherin expression</td>
<td>Cell</td>
<td>43.3</td>
<td>0.2–99.1</td>
</tr>
<tr>
<td>EGFR expression</td>
<td>Cell</td>
<td>45.6</td>
<td>0.2–91.8</td>
</tr>
</tbody>
</table>

### Table III

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Items</th>
<th>Unadjusted HR (95% CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>—</td>
<td>0.94 (0.90–0.99)</td>
<td>.027</td>
</tr>
<tr>
<td>Male sex</td>
<td>—</td>
<td>0.21 (0.03–1.74)</td>
<td>.150</td>
</tr>
<tr>
<td>Cigarette smoking</td>
<td>—</td>
<td>23.36 (0.00–1.22 × 10&lt;sup&gt;5&lt;/sup&gt;)</td>
<td>.471</td>
</tr>
<tr>
<td>Alcohol drinking</td>
<td>—</td>
<td>1.42 (0.31–6.49)</td>
<td>.651</td>
</tr>
<tr>
<td>Betel quid chewing</td>
<td>—</td>
<td>5.85 (1.58–21.72)</td>
<td>.008</td>
</tr>
<tr>
<td>Pathologic T stage</td>
<td>—</td>
<td>1.37 (0.80–2.37)</td>
<td>.255</td>
</tr>
<tr>
<td>Type of cordectomy</td>
<td>—</td>
<td>1.95 (1.22–3.13)</td>
<td>.006</td>
</tr>
<tr>
<td>Bcl-2 expression</td>
<td>cPI (%)</td>
<td>0.97 (0.95–1.00)</td>
<td>.027</td>
</tr>
<tr>
<td></td>
<td>chS</td>
<td>0.99 (0.98–1.00)</td>
<td>.055</td>
</tr>
<tr>
<td>pRB expression</td>
<td>nPI (%)</td>
<td>0.04 (0.00–0.62)</td>
<td>.211</td>
</tr>
<tr>
<td></td>
<td>nHS</td>
<td>0.99 (0.98–1.00)</td>
<td>.304</td>
</tr>
<tr>
<td>p16&lt;sup&gt;NK4A&lt;/sup&gt; expression</td>
<td>cPI (%)</td>
<td>0.99 (0.97–1.00)</td>
<td>.143</td>
</tr>
<tr>
<td></td>
<td>chS</td>
<td>0.99 (0.98–1.00)</td>
<td>.100</td>
</tr>
<tr>
<td>p53 expression</td>
<td>nPI (%)</td>
<td>0.31 (0.02–4.81)</td>
<td>.402</td>
</tr>
<tr>
<td></td>
<td>nHS</td>
<td>1.00 (0.99–1.01)</td>
<td>.388</td>
</tr>
<tr>
<td>c-Myc expression</td>
<td>nPI (%)</td>
<td>0.28 (0.01–6.87)</td>
<td>.437</td>
</tr>
<tr>
<td></td>
<td>nHS</td>
<td>0.99 (0.98–1.01)</td>
<td>.263</td>
</tr>
<tr>
<td>E-cadherin expression</td>
<td>cPI (%)</td>
<td>1.00 (0.97–1.02)</td>
<td>.713</td>
</tr>
<tr>
<td></td>
<td>chS</td>
<td>1.00 (0.99–1.01)</td>
<td>.763</td>
</tr>
<tr>
<td>EGFR expression</td>
<td>cPI (%)</td>
<td>0.99 (0.97–1.02)</td>
<td>.994</td>
</tr>
<tr>
<td></td>
<td>chS</td>
<td>1.00 (0.99–1.01)</td>
<td>.388</td>
</tr>
</tbody>
</table>

<sup>c</sup> = cellular; CI = confidence interval; HR = hazard ratio; HS = histological score; n = nuclear; PI = positive index.

Lee et al.: pRB Expression and DFS in Early Glottic SCC
Kalfert et al.\textsuperscript{31} reported that p16INK4A expression may be a predictor of favorable therapeutic outcomes in nonsmokers with early-intermediate glottic cancer treated with transoral laser microsurgery.\textsuperscript{30} These confounding factors could bias the oncological outcomes described in this study; thus, the results of this study should be interpreted cautiously. However, we aimed to establish a feasible model that can be applied in clinical practice.

### Conclusion

We identified different associations between the expression of pRB and Bcl-2 and disease relapse in patients with early-stage glottic SCC after they had undergone TLM. Age, betel quid chewing, and pRB expression independently predicted 3-year DFS and may help to identify high-risk patients with early-stage laryngeal cancer who can subsequently undergo more intensive follow-up and treatment.

### Bibliography


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**TABLE IV.** Spearman’s Correlation Coefficients Between Variables Included in Multivariate Analyses in the Included Patients.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Adjusted Hazard Ratio (95% CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.94 (0.88-1.00)</td>
<td>0.045</td>
</tr>
<tr>
<td>Betel quid chewing</td>
<td>5.07 (1.32-19.44)</td>
<td>0.018</td>
</tr>
<tr>
<td>Type of cordectomy</td>
<td></td>
<td>Not significant</td>
</tr>
<tr>
<td>Bcl-2 expression</td>
<td></td>
<td>Not significant</td>
</tr>
<tr>
<td>pRB expression</td>
<td>0.02 (0.00-0.28)</td>
<td>0.005</td>
</tr>
<tr>
<td>p16\textsuperscript{INK4A} expression</td>
<td>-</td>
<td>Not significant</td>
</tr>
</tbody>
</table>

CI = confidence interval.

**TABLE V.** Three-Year Disease-Free Survival From Multivariate Analyses in the Included Patients.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Adjusted Hazard Ratio (95% CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.24 (.147)</td>
<td>0.19 (.266)</td>
</tr>
<tr>
<td>Betel quid chewing</td>
<td>0.01 (.952)</td>
<td>0.03 (.880)</td>
</tr>
<tr>
<td>Type of cordectomy</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>Bcl-2 expression</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>pRB expression</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>p16\textsuperscript{INK4A} expression</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Data are expressed as r (P value).


