p16 Influence on Laryngeal Squamous Cell Carcinoma Relapse and Survival

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Abstract

Objective. (1) To identify p16 protein in laryngeal squamous cell carcinoma (LSCC) specimens and to correlate it with the presence of human papillomavirus (HPV) found in these specimens from a previous study. (2) To analyze p16 impact on 10-year overall and disease-free survival.

Study Design. Retrospective case series with oncologic database chart review.

Setting. Academic tertiary care hospital.

Subjects. A total of 123 samples of LSCC (taken from the glottis only) from patients treated with primary surgical resection between 1977 and 2005.

Methods. p16 protein expression was analyzed through immunohistochemistry and compared with the presence of HPV established in our previous studies. Results were compared with histologic, clinicopathologic, and survival parameters, with a 10-year follow-up.

Results. Of the samples, 39.02% were positive for p16, but only 11.38% were positive for both p16 and HPV. The p16+ cohort showed a significant improvement in disease-free survival ($P = .0022$); statistical significance was not achieved for overall survival. p16+ cases had fewer relapses over time, with no relapses after a 2-year follow-up. Age at the time of diagnosis and tobacco consumption were the only epidemiologic factors that influenced overall survival.

Conclusion. The expression of p16 protein was a beneficial prognostic factor for disease-free survival among patients with LSCC of the glottis, with no relapses after a 2-year follow-up.

Keywords

larynx, squamous cell carcinoma, p16, relapse, disease-free survival
according to the size, node involvement, and p16 positivity has been established for oropharyngeal tumors.\(^7\) However, the correlation between p16 overexpression and HPV infection in nonoropharyngeal head and neck sites remains unclear.\(^7\)

Some authors reported an improved survival rate in the p16+ samples.\(^4,8,9\) In addition, the published series rarely follow up patients beyond 5 years, and different modalities of treatment were applied, making these series hard to compare. Furthermore, there are very few laryngeal series with >100 samples.\(^4,7,10\) To date, there is no published series with 10 years of follow-up that specifically correlates the presence of p16 and tumor relapse as an influential survival factor.

The aim of the present study was to determine the presence of p16 protein in specimens from 123 patients with glottic squamous cell carcinoma (g-SCC) and its relationship with overall survival (OS) and disease-free survival (DFS) with a >10-year follow-up. In this study, we also correlate epidemiologic data with the presence of p16 and HPV and their influence on survival.

### Materials and Methods

This study was conducted on specimens from our previous study, which examined whether HPV was identified in patients with laryngeal squamous cell carcinoma who were initially treated surgically for glottic cancer cell carcinoma.\(^11\) Therefore, the inclusion criteria were g-SCCs from patients of both sexes who were initially treated with surgery only (ie, without chemotherapy or radiotherapy). In cases with subsequent recurrent or persistent disease, treatment consisted of salvage surgery, chemotherapy, and/or radiotherapy. Exclusion criteria were as follows: nonglottic tumors, non–squamous cell carcinomas, persistent or recurrent disease at diagnosis, or initial treatment with chemotherapy/radiotherapy only or chemotherapy/radiotherapy and surgery.

In the previous study,\(^11\) 2831 patients were identified between 1977 and 2005 with head and neck neoplasms, of which 847 were patients with g-SCC. A calculated sample size of 209 specimens was required to estimate the prevalence of HPV in g-SCC for an alpha of 0.05, with absolute size of 209 specimens was required to estimate the prevalence of HPV11 globally.\(^4,7,10\) To date, there is no published series with more than 10 years of follow-up that specifically correlates the presence of p16 and tumor relapse as an influential survival factor.

Unfortunately, of the 209 selected samples, 86 were found to be nonviable and therefore unsuitable for the study of p16. Due to technical reasons, it was not possible to carry out an immunohistochemical study with them.

Epidemiological data were obtained from the patients’ clinical records, including demographic, clinical, and tumor data. Tumor relapses were classified as local when in the larynx and nodal when in the cervical nodes.

To evaluate the presence of p16 over time, 4 groups were established according to year of diagnosis. These groups were divided as quartiles (1978-1992, 1993-1995, 1996-1997, 1998-2005) and in equal intervals (1978-1984, 1985-1991, 1992-1998, 1999-2005). To correlate the presence of p16 and HPV, we compared our p16 data with data from a previous study performed on the same population samples that examined the presence of HPV11\(^11\) globally (HPV+ or HPV−) and stratified by oncogenic risk (low or high risk). The oncogenic risk of each serotype of HPV was established according to classification of the International Agency for Research on Cancer.\(^14\) In our previous study,\(^11\) we determined a 22.76% prevalence of HPV in g-SCC. Based on this previous HPV prevalence and the results of p16 of the present study, we established 4 p16/HPV cohorts (HPV+/p16−, HPV−/p16+, HPV+/p16−, and HPV+/p16+).

OS was defined as the time from diagnosis to death from all causes or to the last date of follow-up. DFS was defined as the time from diagnosis to a relapse of disease. During the follow-up, we classified cases of relapses into local or nodal relapses. Given their influence on survival, both groups were compared; thus, the relationship between each group and the presence of p16 was established.

Quantitative variables were expressed as mean, median, and standard deviation as well as interquartile range. These variables were described and compared with qualitative variables via absolute percentage, relative percentage, and the chi-square test. Survival analysis was performed with Cox regression and the Kaplan-Meier method. A log-rank test was used to evaluate whether the differences observed in ≥2 survival curves could be attributed to chance.

### Results

General data included in the study are detailed in Supplemental Material 1, and survival data are included in...
Supplemental Material 2 (available in the online version of the article).

As laid out in the study design, follow-up was at least 10 years, with a median of 18.39 years. All samples were from the glottis, mainly T1, with involvement of a vocal cord (52.03%, n = 64) and without any affected cervical node (N0: 95.93%, n = 118), and so were classified as stage 1 (59.35%, n = 73) according to the American Joint Committee on Cancer criteria.15 Histologic differentiation was not noted in 28.46% (n = 35) of the samples, and most were well-differentiated squamous cell carcinomas (49.59%, n = 61). The mean age at the time of diagnosis was 62.31 years. The most common presenting complaint was dysphonia (96.75%, n = 119). The progression time of the symptoms was collected in most cases (85.36%, n = 105), with a mean evolution time of 8.27 months. Smokers represented 89.43% (n = 111) of the patients, with a mean tobacco consumption of 29.5 cigarettes per day; 61.78% (n = 76) drank alcohol on a daily basis, with a mean alcohol consumption of 2.64 units per day.

Immunohistochemical study showed positivity to p16 in 39.02% (n = 48) of the samples. No significant difference was found for the following comparisons: p16 positivity and age at diagnosis (P = .31), the presenting complaint (P = .19), the evolution time of the symptoms (P = .66), tobacco use (P = .78), alcohol consumption (P = .79), the primary site of the tumor (P = .66), g-SCC histologic subtype (P = .93), size (P = .14), node involvement (P = .89), and tumor stage (P = .077).

There was no significant variation of p16 positivity over time, based on the established temporary cohorts in terms of quartiles (P = .6) or equal intervals (P = .086).

Relationship between p16 and HPV

Only 14 of 123 samples (11.38%) were p16+ in cases previously determined to be HPV+.11 The rest of the samples were HPV-/p16− (49.59%, n = 61), HPV-/p16+ (27.64%, n = 34), and HPV+/p16− (11.38%, n = 14). There was no significant relationship between p16 and HPV—generally (HPV+ or HPV−; P = .18) and by oncogenic risk (high or low risk; P = .067).14

No significant difference was found in comparisons between any p16/HPV status and the following: age at the time of diagnosis (P = .61), tobacco use (P = .77), alcohol consumption (P = .84), presenting complaint (P = .24), evolution time of symptoms (P = .87), tumor size (P = .74), and histologic subtype of g-SCC (P = .69).

A significant relationship was found between the p16−/HPV− group and tumor size (P = .012). It is noteworthy that 75% of the total of T4 tumors were p16−/HPV−. Similar to the findings on tumor size, a significant relationship between tumor stage and p16 negativity and HPV negativity was established (P = .02). Of the total of stage IV tumors, 69.32% were assigned to the p16−/HPV− cohort. Statistical significance was not established between any cohort and node involvement at time of diagnosis (P = .09).

Disease-Free Survival and Overall Survival

The presence of p16 was a decisive factor on DFS (P = .0022), showing a hazard ratio (HR) of 0.2432 (P = .0008): the p16+ group had a 76% lower chance of death per year (Figure 1). Furthermore, DFS remained stable between the second and at least the 10th year of follow-up in the p16+ group.

There was a significant relationship between p16+/HPV+ cohorts and DFS (P = .0021), presenting the best rate of DFS. Statistical significance was not achieved in DFS relative to age at the time of diagnosis (P = .84), presenting complaint (P = .33), evolution time of symptoms (P = .42), tobacco consumption (P = .44), alcohol consumption (P = .53), primary localization (P = .9), histologic type (P = .34), tumor size (P = .13), node involvement (P = .34), or tumor stage (P = .07). Statistical significance was also not achieved in OS relative to the presence of p16 (P = .77), tumor histology (P = .8), primary tumor site (P = .31), size (P = .57), node involvement (P = .78), or tumor stage (P = .32).

There was a significant relationship between the presenting complaint and OS (P = .0441), but no HR could be set for each symptom at the time of diagnosis. Patients
ever, did not have a decisive impact on OS (previous and recent studies). However, in our series, between the presence of p16 and HPV in g-SCC, similar to

In our study, there is no statistically significant relationship between the presence of p16 and HPV in g-SCC, similar to previous and recent studies. Considering that one of the limitations of our study was the impossibility of analyzing 86 nonviable samples. Despite this lessering of the total number of specimens, the sample selected on the basis of HPV prevalence studies is one of the largest published to date. Considering all presented, we believe that it is necessary to carry out prospective studies to ensure the viability of all samples and to avoid the loss of information; moreover, using specific methods (eg, genetic or epigenetic) could help solve this issue.
Liang et al.⁸ found that positive p16 immunostaining alone was not associated with significantly improved survival, but they agreed that p16+ samples present a better OS,⁶,⁹ unlike other studies.¹⁰,¹⁶,¹⁷ In our study, there was no difference between p16 cohorts regarding OS, similar to previous studies.⁷,¹⁰

In the few studies that showed DFS rates regarding the presence of p16, there was wide variability and an absence of statistical significance. Some of these studies reported a DFS rate that is similar in both p16+ and p16− cohorts;⁷ others showed a better DFS in the p16− cohort;⁶ and some even reported a better DFS in the p16+ group.⁴ This variability in the data can be explained by the follow-up time used. In all these studies, the median follow-up time was no longer than 7 years,⁴,⁷,¹⁰ far from our median follow-up time of 18.39 years. Based on this follow-up time, our study shows the significant association between p16 positivity and a better DFS rate and 76% less chance of death per year within the p16+ group.

From the epidemiologic factors studied, only age at the time of diagnosis and tobacco consumption were influential factors on OS. Thus, every year at the time of diagnosis, the chance of death increased by 3.76%. Most of the previous studies on this topic did not directly address the relationship between survival rate and age at the time of diagnosis, assuming a worse survival rate among the older patients.²⁴,²⁵ Megwalu and Sikora²⁶ specifically investigated this relationship, confirming the influence of age at the time of diagnosis on OS with an almost identical HR (1.03) as in the present study. However, every cigarette per day that the patient reported smoking increased the chance of death by 2.86%. This finding had already been described in previous studies, adding a multiplicative effect with alcohol consumption.²⁷ This effect was not confirmed in our study, where tobacco consumption and alcohol consumption were shown as independent factors. In our study, no other epidemiologic and/or tumor factors were determined in the survival analysis.

Table 1. p16 Detection Frequencies in Laryngeal Squamous Cell Carcinoma: Studies with Follow-up and Survival Description Specification.

<table>
<thead>
<tr>
<th>Study</th>
<th>Laryngeal Specimens, n</th>
<th>p16, % (n)</th>
<th>Follow-up, mo</th>
<th>Better Survival in . . .</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chernock⁹ (2013)</td>
<td>76</td>
<td>28 (21)</td>
<td>34.6 (mean)</td>
<td>p16+</td>
<td>.058 (OS)</td>
</tr>
<tr>
<td>Chung⁴ (2014)</td>
<td>181</td>
<td>17.1 (31)</td>
<td>Not specified (maximum, 60)</td>
<td>p16+</td>
<td>.08 (OS), .11 (DFS)</td>
</tr>
<tr>
<td>Salazar¹⁶ (2014)</td>
<td>50</td>
<td>24 (12)</td>
<td>67 (median)</td>
<td>p16− (OS and DFS)</td>
<td>.8916 (OS), .80 (DFS)</td>
</tr>
<tr>
<td>Larque¹⁷ (2015)</td>
<td>45</td>
<td>9 (4)</td>
<td>48 (median)</td>
<td>p16− (OS)</td>
<td>.84 (OS)</td>
</tr>
<tr>
<td>Young⁷ (2015)</td>
<td>307</td>
<td>6.5 (20)</td>
<td>41 (median)</td>
<td>Similar (OS and DFS)</td>
<td>.65 (OS), .22 (DFS)</td>
</tr>
<tr>
<td>Hernández¹⁰ (2016)</td>
<td>101</td>
<td>7.9 (8)</td>
<td>Not specified (maximum, 60)</td>
<td>Similar (OS)</td>
<td>.84 (OS)</td>
</tr>
</tbody>
</table>

Abbreviations: DFS, disease-free survival; OS, overall survival.

Conclusion

The expression of p16 protein is a beneficial prognostic factor for survival of patients with laryngeal squamous cell carcinoma. These patients have an increased DFS rate and fewer relapses, with no relapses occurring after 2 years of follow-up. Age at the time of diagnosis and tobacco consumption were shown to be the only epidemiologic factors that influence OS.

Acknowledgments

We are grateful to our friend and colleague Dr. Brandáriz Castelo, whose lifetime of work made an essential contribution to this study. We hope that you are proud of it. We are also grateful to Dr Ballestín Carcavilla for his huge amount of work on all the pathologic samples and to David Lora Pablos for his exhaustive statistical efforts and plentiful advice in the development of this manuscript.

Author Contributions

Alvaro Sánchez Barrueco, design of the study, selection process, data analysis, acquisition and analysis of results, draft the manuscript, final approval of the version to be published, accountability for all aspects of the work; Fernando González Galán, acquisition of data, data analysis, drafting the manuscript, final approval of the version to be published, accountability for all aspects of the work; José Miguel Villacampa Aubía, substantial contributions to the design of the work, revision of the manuscript, data analysis, drafting the manuscript, final approval of the version to be published, accountability for all aspects of the work; Gonzalo Díaz Tapia, substantial contributions to the conception of the work, revision of the manuscript, data analysis, drafting the manuscript, final approval of the version to be published, accountability for all aspects of the work; Cristina Martín-Arriscado Arroba, substantial contributions to the design of the work, revision of the manuscript, data analysis, drafting the manuscript, final approval of the version to be published, accountability for all aspects of the work; Carlos Cáceres Gutiérrez, participating in the design of the study, statistical analysis, data analysis, drafting the manuscript, final approval of the version to be published, accountability for all aspects of the work; Carlos Almodóvar Álvarez, design of the study, data analysis, drafting the manuscript, final approval of the version to be published, accountability for all aspects of the work; Carlos Almodóvar Álvarez, design of the study, data analysis, drafting the manuscript, final approval of the version to be published, accountability for all aspects of the work.

Disclosures

Competing interests: None.

Sponsorships: None.
Funding source: Fundación Mutua Madrileña provided the funds necessary to conduct studies of pathology. None of the authors received any funding.

Supplemental Material
Additional supporting information is available in the online version of the article.

References