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ORIGINAL ARTICLE

Combination of p16 levels and pre-radiotherapy factors predicts outcome in patients treated for oropharyngeal carcinoma

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Summary

Purpose: *To explore whether tumor biomarkers and pre-treatment factors correlate with treatment outcome in patients with oropharyngeal squamous cell carcinoma (SCC).*

Methods: *Fifty-seven consecutive patients diagnosed with oropharyngeal SCC were treated using intensity modulated radiotherapy (IMRT). Thirty-four (60%) patients were treated with definitive chemoradiotherapy to a median total dose of 70 Gy and 23 (40%) were treated with postoperative RT to a median total dose of 66 Gy. Concurrent platinum-based chemotherapy was used in 51 patients (90%) and cetuximab in 3 (5%) patients.*

Results: *Forty-four (77%) cases were positive and 13 (23%) were negative for p16 expression. Eighty-eight percent of non-smokers, 87% of smokers in their remote past and 56% of active smokers were diagnosed with p16-positive cancer. After 22 months median follow up, 51 (89%) patients were alive. Forty-five (77%) patients were without evidence of disease at their last follow up, 82% of the patients with p16-positive tumors vs 58% of those with p16-negative cancer, respectively (p= 0.04). Locoregional disease-free survival was 82% for the entire cohort, 91% for patients treated postoperatively and 76% for patients treated with definitive chemoradiotherapy. Five (9%) patients developed distant metastases, and 3 (5%) developed new malignancies. One third of the patients with pre-RT hemoglobin level of ≤ 11 g/dL experienced persistent/recurrent disease; 80% of patients with hemoglobin ≤ 11 g/dL were smokers and 42% had p16-negative tumors.*

Conclusions: *Smoking, p16 expression and pre-RT anemia are interrelated and influence outcome in oropharyngeal cancer patients and should be evaluated as stratifying variables in further clinical trials.*

Key words: *anemia, oropharyngeal carcinoma, p16 level, smoking*

Introduction

Tobacco and alcohol consumption represent the primary risk factors for head and neck squamous cell carcinoma (HNSCC). However, it has recently become evident that infection with high-risk human papillomavirus (HPV) is etiologically linked with a subgroup of cancers of the head and neck region, especially the oropharynx [1]. Numerous retrospective and prospective studies have demonstrated that among patients with oropharyngeal SCC, patients with HPV-positive tumors have a better prognosis than patients with HPV-negative tumors [2-9].

Anemia is common in cancer patients and has been shown to adversely impact the prognosis of patients treated with RT for HNSCC [10-21]. Anemia might be caused by a direct interference by the malignancy in red blood cell production or by an indirect effect of high tumor burden and/or tumor-related cytokines (interleukin-1, interferon gamma, and tumor necrosis factor), which have been recognized to induce hemolysis, suppression of erythropoiesis, and impaired response of erythroid progenitor cells to erythropoietin [13].

The present study explored the correlation between the tumor biomarkers and pre-treatment factors and treatment outcome in patients with

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oropharyngeal carcinoma. The impact of HPV-associated tumor cell p16 expression, smoking and anemia were analyzed.

Methods

Patient selection

Between August 2006 and January 2011, 57 consecutive patients with histologically confirmed SCC of the oropharynx (21 patients with tonsillar cancer, 33 with base of tongue cancer, and 3 with pharyngeal wall tumors) were treated at our institution. After obtaining institutional review board approval, the patient records were reviewed, including pre- and postoperative records, operative reports, pathology records, radiological studies before and after treatment, smoking history, comorbidities (including coronary artery disease, hypercholesterolemia, diabetes mellitus, chronic obstructive pulmonary disease and hypertension), pre-RT hemoglobin and albumin levels. For the purposes of this analysis, remote history of smoking was defined as ≥ 3 years from the time the patients quit smoking.

Patient characteristics are summarized in Table 1. Pretreatment evaluation included complete history and physical exam with fiberoptic nasopharyngolaryngoscopy, complete blood counts, basic metabolic panel and liver function tests, radiological imaging with computed tomography (CT) and/or magnetic resonance imaging (MRI), and dental evaluation.

Radiotherapy

All patient cases were reviewed in a multidisciplinary tumor board to determine the primary treatment course with surgery vs definitive chemoradiotherapy. Patients undergoing primary surgical treatment were treated with postoperative RT alone or adjuvant chemoradiotherapy based on pathological risk factors. RT was delivered using IMRT with a simultaneous in-field boost technique with 6 MV photons. Patients receiving definitive RT were treated to a median dose of 70 Gy (range, 69.96-72). Patients treated with adjuvant RT were treated to a median total dose of 66 Gy (range, 54-66). The median time from surgery to the start of postoperative RT was 38 days (range, 29-58). Nine (16%) of 57 patients had >3 days radiation therapy treatment delay, due to severe acute treatment-related toxicity (mucositis and/or dermatitis).

Surgery

Twenty-three (40%) patients were treated with postoperative RT after primary tumor resection and selective cervical lymph node dissection. Seven (12%) of 23 patients treated with postoperative RT had nodal extracapsular extension documented in the neck dissection specimens, and 8 (14%) had positive surgical margins. Nineteen (33%) of 34 patients treated with definitive chemoradiotherapy underwent a pre-RT cervical lymph node dissection, and in 7 (12%) cases the neck dissection performed was bilateral. One patient treated with definitive chemoradiotherapy underwent bilateral neck dissection due to clinical and radiological nodal residual disease after the end of chemoradiotherapy; all cervical lymph nodes dissected in this case were negative for malignancy.

Table 1. Patient characteristics

Characteristics	*N (%)*
Gender	
Male	48 (84)
Female	9 (16)
Primary site	
Tonsillar SCC	21 (37)
Base of tongue SCC	33 (58)
Pharyngeal wall	3 (5)
Smoking status	
Non-smoker	17 (30)
Active smoker	16 (28)
Remote history of smoking	24 (42)
HPV status (p16 expression)	
Positive	44 (77)
Negative	13 (23)
Stage	
III	3 (14)
IVA	42 (74)
IVB	7 (12)
Pre-radiotherapy hemoglobin level (g/dL)	
≤ 11	15 (26)
> 11	42 (74)
Type of radiotherapy	
Post-operative	23 (40)
Definitive	34 (60)

SCC: squamous cell carcinoma, HPV: human papillomavirus

Chemoradiotherapy

Thirty-four (60%) patients were treated with definitive RT. Concurrent platinum-based chemotherapy was used in 51 patients (90%) and cetuximab in 3 (5%). All patients treated with definitive RT received concurrent platinum-based chemotherapy.

p16 immunohistochemistry staining protocol

Immunohistochemistry staining for p16 was performed on representative 4-μm sections cut from formalin-fixed, paraffin-embedded tissue blocks using a monoclonal antibody to p16 (MTM laboratories before 2012, Ventana starting in 2012; monoclonal; 1:1 dilu-

tion) on a Ventana Benchmark LT automated immunostainer (Ventana Medical Systems, Inc., Tucson, AZ) according to standard protocols. Detection involved Ventana's Ultraview Universal DAB Detection kit that uses a cocktail of enzyme-labeled secondary antibodies that locate the bound primary antibody. The complex is then visualized with hydrogen peroxide substrate and a 3:30-diaminobenzidine tetrahydrochloride (DAB) chromogen. No biotin is involved. Antigen retrieval, standard on the machine, used the Ventana CC1, EDTA-TRIS, pH 8.0 solution. A known p16 expressing head and neck squamous cell carcinoma case was used as positive control and sections of normal tonsil were used for negative controls with each run. Staining was nuclear and cytoplasmic and was graded by a single pathologist binarily into positive (more than 75% of tumor cells positive, nuclear and cytoplasmic staning) and negative (staining in less than 75% of tumor cells) groups.

Statistics

Continuous variables were summarized using medians and ranges. Categorical variables were summarized with frequencies and percentages. Groups were compared using the Kruskal-Wallis test for continuous variables and Pearson's chi-square test for categorical variables. Differences in locoregional disease-free survival were evaluated using the log-rank test. Locoregional control was determined using the Kaplan-Meier method. Cox proportional hazards regression analysis was used to determine independent prognostic factors correlated with locoregional disease-free survival. Covariates were included in the model if their inclusion changed the log hazard ratio for p16 by ±15%. All p-values were two-sided and a p-value of <0.05 was considered statistically significant.

Results

Patient and treatment characteristics

Forty-eight (84%) patients were men and 9 (16%) were women. Median age at the time of diagnosis was 58 years (range 37-77). Forty-four (77%) patients were diagnosed with a p16 positive cancer and 13 (23%) with a p16 negative cancer.

Smoking history

Seventeen (30%) patients were non-smokers in this cohort, 24 (42%) patients reported a remote history of smoking and 16 (28%) patients were actively smoking at the time of primary treatment of their oropharyngeal cancer.

Relationship between smoking, pretreatment anemia and p16 status

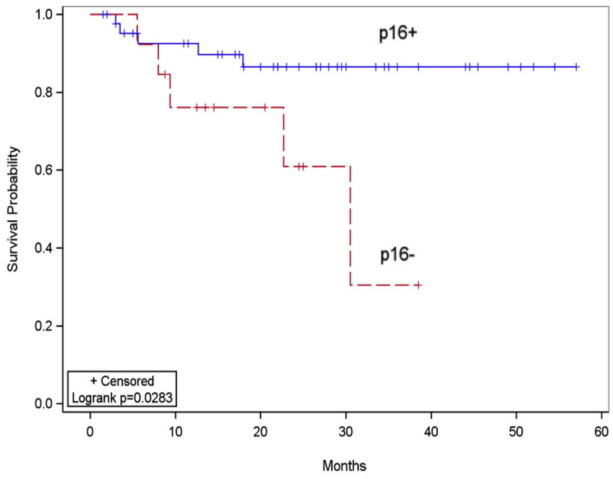

Figure 1. Disease-free survival correlated with tumor p16 level.

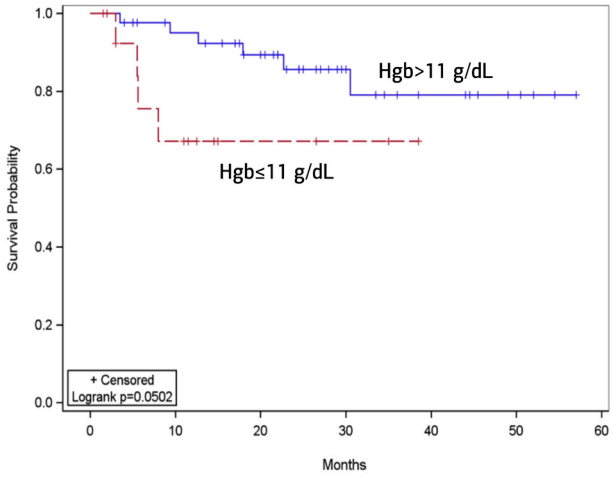

Figure 2. Locoregional disease-free survival correlated with pre-radiotherapy hemoglobin level.

The relationship between smoking, pretreatment anemia and p16 status is presented in Table 2. Eighty-eight percent of non-smokers and 87% of the patients with a remote history of smoking were found to have a p16-positive tumor as compared to only 56% of active smokers (p=0.04). There was no statistically significant difference between the median age at the time of diagnosis of 57.5 years for patients diagnosed with p16-positive tumors vs 58 years age for patients diagnosed with p16-negative tumors. Moreover, no difference in the incidence of comorbidities has been seen between different subgroups of patients diagnosed with p16-positive or negative tumors.

After a median follow up of 22 months (range 2-49), 51 (89%) patients were alive. Forty-five (77%) patients were without evidence of disease at their last follow up; 82% of the patients with p16-positive tumors vs 58% of the patients with

Table 2. Association of patient characteristics with p16 status, anemia, and smoking status

Variable	*p16 status*		*Anemia*		*Smoking status*			
	Positive %	*p-value*	*Anemic %*	*p-value*	*Non-smoker*	*Quit smoking*	*Active smoker*	*p-value*
Gender								
Female	55.6	0.1872	44.4	0.2230	33.3	11.1	55.6	0.0722
Male	81.3		22.9		29.8	46.8	23.4	
Nodal extracapsular extension								
No	75.0	0.7101	29.5	0.4779	30.2	37.2	32.6	0.4805
Yes	84.6		15.4		30.8	53.8	15.4	
p16 status								
p16+			22.7		34.1	45.5	20.5	0.0432
p16-			38.5		16.7	25.0	58.3	
Margin status								
Negative	66.7	0.6822	0.0	0.0457	41.7	41.7	16.7	0.4566
Not applicable	78.8		36.4		21.9	40.6	37.5	
Positive/close	83.3		25.0		41.7	41.7	16.7	
Site of disease								
Base of tongue	78.8	0.0089	36.4	0.1180	28.1	43.8	28.1	0.5678
Pharyngeal wall	0.0		0.0		0.0	33.3	66.7	
Tonsil	85.7		14.3		38.1	38.1	23.8	
Smoking								
Active smoker	56.3	0.0432	37.5	0.2428				
Non-smoker	88.2		11.8					
Quit smoking	87.0		26.1					
T stage								
1	71.4	0.3797	0.0	0.0210	28.6	42.9	28.6	0.5353
2	81.8		13.6		36.4	40.9	22.7	
3	64.7		35.3		37.5	25.0	37.5	
4	90.9		54.5		9.1	63.6	27.3	
Anemia								
0	81.0	0.2944			35.7	40.5	23.8	0.2428
1	66.7	.			14.3	12.9	42.9	

p16-negative cancer (p=0.028, Figure 1). Locoregional disease-free survival was 82% for the whole group, 91% for patients treated postoperatively and 76% for patients treated with definitive chemoradiotherapy. Ten (18%) patients demonstrated locoregional failures or had persistent disease (8 patients treated with definitive chemoradiotherapy and 2 treated with postoperative RT). Five (9%) patients developed distant metastases. Three of 5 patients who developed distant metastases were treated for lung or bone oligometastases and were free of disease more than 1 year following treatment for metastatic disease. Three (5%) patients developed new primary malignancies.

After a median follow up of 22 months, one third of patients with pre-RT hemoglobin level of ≤ 11 g/dL experienced persistent/recurrent disease, as compared to only about 10% of patients with a hemoglobin >11 g/dL (p=0.05, Figure 2)). Eighty percent of the patients with anemia ≤ 11 g/dL were smokers and 42% had p16-negative tumors. Pre-RT hemoglobin level of ≤ 11 g/dL was more frequent in patients with present or remote history of smoking than in never smokers (33 vs 12%; p=0.04).

Discussion

There is a growing body of evidence demonstrating that HPV-positive HNSCC represents a separate biologic subgroup, distinct from those HNSCC that are induced by tobacco and alcohol

[1,4,6-8]. Recent studies have indicated that patients with HPV-positive HNSCC have a favorable prognosis when compared with patients whose tumors are HPV-negative [2-9]. The expression of p16 is an established biomarker for the function of the HPV E7 oncoprotein [4,5,8,22,23]. The p16 expression assay is not specific for HPV type, hence p16 expression status is a very good surrogate for tumor HPV status. Our results corroborate with the previously published data suggesting that tumors' positivity for p16 is significantly correlated with noticeably improved locoregional control. Seventy-seven percent of the patients in this study were without evidence of disease at their last follow up, 82% of patients with p16-positive tumors vs 58% of patients with p16-negative cancer, respectively (p= 0.03).

Epidemiologic data has demonstrated that HPV-positive HNSCC appears to be associated with lower exposure to tobacco and with younger age at the time of diagnosis, factors that may influence positively the prognosis by themselves, regardless of tumor biology [1,4,7]. The results of the present study are in accordance with previously published data demonstrating a higher incidence of p16-positive tumors between nonsmokers or patients reporting a remote history of smoking. Eighty-eight percent of non-smokers, 87% of smokers in their remote past and only 56% of active smokers were diagnosed with p16-positive cancer.

Patients diagnosed with HPV-positive HNSCC could potentially have less comorbidities and a different risk profile in term of their age and exposure to tobacco when compared with patients with HPV-negative tumors. However, there was no statistically significant difference between the median age at the time of diagnosis for nonsmokers vs patients actively smoking in the current study or between patients diagnosed with p16-negative vs p16-positive tumors. Moreover, no difference in the incidence of comorbidities has been seen between different subgroups of patients diagnosed with p16-positive or negative tumors.

Our results suggest that anemia is an independent predictor of decreased disease-free survival and higher rate of late treatment related complications. Patients with pre-RT hemoglobin level of ≤ 11 g/dL experienced a higher rate of persistent/recurrent disease when compared with patients with pre-RT level of > 11 g/dL. These results corroborate with previously published data [10]. The reasons for this phenomenon remain to be determined. It has been hypothesized that tumors in patients with low hemoglobin serum levels might restrain a significant number of tumor cells clones which are biologically more aggressive [13]. Furthermore, the association between anemia and adverse outcome may be mediated through tumor hypoxia. Tumor hypoxia has been shown to be associated with poorer treatment outcome in patients with head and neck cancer [24,25]. Despite the fact that tumor hypoxia and anemia represent powerful predictors of patient outcome, cause-and-effect link between these two prognostic factors is far more vague [26]. Previous studies have found only weak associations between anemia and tumor hypoxia or hypoxic tumor subvolumes [24,27]. Most tumors in anemic patients had elevated hypoxic sub-volumes, but a number of tumors in non-anemic patients also had elevated hypoxic sub-volumes [24,27]. Furthermore, several studies have reported that RT was more effective under well-oxygenated conditions than under hypoxic or anoxic conditions [17,28-31]. There is currently limited published data regarding the significance of pretreatment anemia as a prognostic factor in HNSCC patients treated with concurrent chemoradiotherapy [26,32].

Moreover, there is currently no evidence that correction of anemia improves prognosis for head and neck cancer patients [33,34]. Hemoglobin level should be considered as a stratifying variable in future clinical trials rather than a factor to be targeted itself and should be further evaluated.

Our results suggest that there is an interrelation between anemia and smoking in patients with head and neck carcinoma. Pre-RT hemoglobin level of ≤ 11 g/dL was more frequent in patients with present or remote history of smoking than in never smokers (33 vs 12%, respectively).

Conclusion

Smoking, p16 expression and pre-RT anemia seem to be interrelated and influence outcome in patients with oropharyngeal cancer and should be evaluated in future prospective clinical trials.

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