Impact of p16 expression in oropharyngeal cancer in the postoperative setting: The necessity of re-evaluating traditional risk stratification

Jeongshim Lee

Department of Medicine

The Graduate School, Yonsei University

Impact of p16 expression in oropharyngeal cancer in the postoperative setting: The necessity of re-evaluating traditional risk stratification

Directed by Professor Ki Chang Keum

The Master's Thesis
submitted to the Department of Medicine,
the Graduate School of Yonsei University
in partial fulfillment of the requirements for the degree
of Master of Medical Science

Jeongshim Lee

June 2016

This certifies that the Master's Thesis

of Jeongshim Lee is approved.

Thesis Supervisor: Ki Chang Keum

Thesis Committee Member#1: Se-Heon Kim

Thesis Committee Member#2: Sang Joon Shin

The Graduate School
Yonsei University

June 2016

ACKNOWLEDGEMENTS

First of all, I would like to thank my supervisor, Professor Ki Chang Keum, for giving me such inspiring advice and support for preparing this thesis and setting a good example for me by devoting himself to patients suffering from cancer. Also I would like to express my gratefulness to professor Se-Heon Kim and Sang Joon Shin for giving me a great amount of advice essential for completing this article. I would like to appreciate to professor Chang-Ok Suh, Jinsil Seong, Chang Geol Lee, Jaeho Cho, Yong Bae Kim, Ik Jae Lee, Woong Sub Koom, and Jun Won Kim for always offering the great instructions with careful concern.

Jeongshim Lee

<TABLE OF CONTENTS>

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

Impact of p16 expression in oropharyngeal cancer in the postoperative setting: The necessity of re-evaluating traditional risk stratification

Jeongshim Lee

Department of Medicine

The Graduate School, Yonsei University

(Directed by Professor Ki Chang Keum)

Purpose: To evaluate the impact of p16 expression as a surrogate marker of human papillomavirus status in oropharyngeal squamous cell carcinoma (OPSCC) patients underwent surgery followed by postoperative radiotherapy (PORT).

Patients and Methods: We identified 126 consecutive patients with histologically confirmed, newly diagnosed OPSCC who received surgery followed by RT, and had p16 expression data available. All patients were treated between 2001 and 2011. Patients with high risk factors (positive surgical margin and/or extracapsular extension) or other risk factors (multiple positive lymph nodes, perineural/lymphovascular invasion) were offered PORT with or without concurrent chemotherapy.

Results: One hundred and four (82.5%) patients were p16-positive (p16 (+)) and 22

(17.5%) were p16-negative (p16 (-)). With a median follow-up of 56 months, patients with p16 (+) OPSCC exhibited a significantly better 5-year disease-free survival (DFS) (80.7% vs. 57.6%, $p < 0.001$) and overall survival (OS) (84.9% vs. 59.1%, $p < 0.001$) than those with p16 (-) tumors. The p16 (+) OPSCC with high risk factors (n=64) showed no difference in DFS (79.7% vs. 68.3%; $p = 0.531$) and OS (82.1% vs. 76.2%; $p = 0.964$) between PORT and PORT with concurrent chemotherapy.

Conclusion: Expression of p16 is a strong, independent prognostic factor of survival in the postoperative setting of OPSCC. The favorable prognosis of p16 (+) OPSCC suggests a need to re-examine traditional risk stratification for determining optimal adjuvant treatment.

Key words: oropharyngeal squamous cell carcinoma; HPV; p16; postoperative setting; risk stratification.

Impact of p16 expression in oropharyngeal cancer in the postoperative setting: The necessity of re-evaluating traditional risk stratification

Jeongshim Lee

Department of Medicine

The Graduate School, Yonsei University

(Directed by Professor Ki Chang Keum)

I. INTRODUCTION

Over several decades, there has been a change in the etiology of oropharyngeal squamous cell carcinoma (OPSCC). Overall, this change may be attributed to decreased smoking and alcohol consumption and increased infection of human papillomavirus (HPV) [1-3]. An estimated 70% of all OPSCCs are due to HPV. In particular, cancers of the palatine tonsils and the base of tongue may be up to 90% HPV-positive [2,4].

The presence of HPV infection in OPSCC is a major determinant in prognosis. Generally, patients with HPV-positive OPSCC have a superior outcome compared with patients with HPV-negative disease [3,5-7]. However, this favorable prognosis of OPSCC patients with HPV-positive have been demonstrated in clinical

studies that were focused on patients treated by primary radiotherapy (RT) or chemoradiotherapy (CRT). Therefore, the report of patients with HPV associated OPSCC treated with surgical resection followed by postoperative RT (PORT) is relatively decimal.

In our institution, the application of surgery followed by PORT for OPSCC is the preferred course of treatment. It has been used increasingly under the influence of recent advances in robotic and trans-oral laser microsurgery. We evaluated the clinical outcome of patients with HPV associated OPSCC based on p16 expression as surrogate marker of HPV status in the oropharynx who underwent surgical resection followed by PORT

II. MATERIALS AND METHODS

1. Study population

We identified 150 consecutive patients with histological confirmed, newly diagnosed, and previously untreated OPSCC who received surgery followed by PORT with curative intent at our institution between January 2001 and December 2011. All clinical and pathological data regarding disease and treatment characteristics were reviewed. This retrospective review was approved by the Institutional Review Board. Five patients who received induction chemotherapy before surgical resection were excluded. And we excluded 19 patients with unavailable HPV tumor status via p16 immunohistochemical analysis. The 126 remaining patients were included for analysis.

All 126 patients underwent surgical resection for OPSCC. In our institution, trans-oral robotic surgery (TORS) has not been used commonly until 2011, although TORS was first applied in 2008. Based on final pathologic reports after surgery, patients with risk factors such as incomplete resection, extracapsular extension (ECE), close/positive surgical margin, multiple positive lymph node, perineural invasion (PNI), and lymphovascular invasion (LVI)) were offered PORT. Since 2007, concurrent chemotherapy has been generally administered to patients with high risk factors (positive surgical margin and/or ECE) and other combined risk factors, according to the clinical discretion of physician. RT doses for high risk areas (residual tumor, positive surgical margin area, positive lymph node bed, especially with ECE, and unresectable positive retropharyngeal lymph node) were 60-70 Gray (Gy). The

doses for intermediate risk areas (positive tumor bed area, positive lymph node area, and minimum of first nodal echelons beyond positive lymph node area) were 54-60 Gy. The doses for low risk areas (contralateral neck node, lower neck) were 50-54 Gy.

2. Immunohistochemistry for p16

Pathological review was carried out by two pathologists who specialize in head and neck cancer. Eligible samples included histopathologically confirmed invasive OPSCC, and 126 tumor tissues could be retrieved from the pathology archives. To assess HPV status of each tumor, we examined p16 expression, which is recognized as a surrogate marker for HPV in the oropharynx, using formalin-fixed, paraffin-embedded (FFPE) surgical tissue. The p16-immunostaining was carried out with a CINtec TM Histology Kit (Roche MTM laboratories AG, Heidelberg, Germany), which contains the mouse monoclonal antibody INK4A that recognizes p16. Representative 4-μm tumor sections cut from FFPE tissue blocks were de-paraffinized. After heat-induced epitope retrieval, immunohistochemistry for $p16^{INK4a}$ was performed with a primary antibody dilution of 1:7 per manufacturer's protocol. Samples were considered p16-positive (p16 (+)) if strong and diffuse nuclear and cytoplasmic immunostaining was observed in at least 70% of the carcinoma tissue. Tissues with only faintly diffuse or no reactivity were considered to be p16-negative (p16 (-)).

3. Statistical analysis

Disease-free survival (DFS) was defined as the amount of time from the start of treatment to the date of any disease recurrence or death from any cause. Overall survival (OS) was calculated as the amount of time from the start of treatment to the date of death from any cause or last day when the patient was known to be alive. OS and DFS were estimated using the Kaplan–Meier method and survival curves were compared using the log-rank test. To determine the effects of distinct prognostic factors on survival, multivariate analysis was performed according to the Cox's regression model in a stepwise backward elimination method. Differences in patient characteristics between p16 (+) and p16 (−) tumors were assessed using the Pearson χ^2-test. In all statistical analyses, $p < 0.05$ was considered to be significant. Statistical analysis was carried out using IBM SPSS Statistics version 20 (SPSS Inc., Chicago IL, USA).

III. RESULTS

1. Patient, tumor, and treatment characteristics

Of the 126 patients with established p16 status, 104 (82.5%) were p16 (+) and 22 (17.5%) were p16 (−). Patients that were p16 (+) more commonly complained of a neck mass as initial symptom, whereas those that were p16 (−) had symptoms related to the primary tumor site ($p = 0.017$). Patients that were p16 (+) had tumors confined to the tonsil or base of tongue, while p16 (−) patients had tumors in all oropharyngeal sites ($p < 0.001$). The distribution of age at diagnosis ($p = 0.621$) and smoking history ($p = 0.536$) were not significantly different between the two cohorts. With respect to tumor characteristics, there were no significant differences in the distribution of risk factors between both groups. The patient and tumor characteristics were summarized in Table 1.

An open surgical approach was undertaken in 85.7%, whereas 14.3% underwent transoral robotic surgery. All patients, except for one, underwent neck dissection. PORT after surgical approach was offered to 116 patients with risk features of a close/positive margin, ECE, PNI, LVI, and multiple positive lymph nodes, while the remaining 10 patients received PORT at the discretion of the physician. Of these 10 patients, nine had a primary tumor larger than 3cm, and one had a pathological T4a tumor. Among all patients, 89 (70.6%) patients received PORT alone and 37 (29.4%) patients received PORT with concurrent chemotherapy or targeted agent. PORT was delivered by 3-dimensional conformal RT in 27 patients (21.4 %) and by intensity-

modulated radiation therapy in 99 patients (78.6%). Of the 37 patients (40%) with PORT and concurrent chemotherapy, 34 received cisplatin-based chemotherapy, whereas two received cetuximab and one received TS-1. The detailed treatment characteristics are shown in Table 2. In addition, we summarized the characteristics of the patients according to adjuvant therapy (PORT vs. PORT with chemotherapy) in Table 3. Distributions for each risk factor and combined risk factors were significantly different between patient groups receiving both adjuvant treatments

Table 1. Patient and tumor characteristics

Variables		All, N=126	p16 expression		*p*
			p16 (+), N=104	p16 (-), N=22	
Age, years	Median (range)	58 (32-78)	58 (32-78)	62 (41-74)	
Age group, n (%)	<60 years	69 (54.8)	58 (55.8)	11 (50.0)	0.621
	≥60 years	57 (45.2)	46 (44.2)	11 (50.0)	
Sex, n (%)	Male	110 (87.3)	89 (85.6)	21 (95.5)	0.206
	Female	16 (12.7)	15 (14.4)	1 (4.5)	
Subsite, n (%)	Tonsil	10 (80.2)	89 (85.6)	12 (54.5)	<0.001
	Base of tongue	19 (15.1)	15 (14.4)	4 (18.2)	
	Soft palate	4 (3.2)		4 (18.2)	
	Posterior wall	2 (1.6)		2 (9.1)	
Initial Symptoms, n (%)	Neck mass	63 (50.0)	57 (54.8)	6 (27.3)	0.017
	Sore throat/dysphagia	42 (33.3)	29 (27.9)	13 (59.1)	
	Tonsil lesion	21 (16.7)	18 (17.3)	3 (13.6)	
Smoking group, n (%)	Never	75 (59.5)	60 (57.7)	15 (68.2)	0.536
	<10 PY	3 (2.4)	3 (2.9)		
	≥10 PY	48 (38.1)	41 (39.4)	7 (31.8)	
ECOG, n (%)	0~1	122 (96.8)	100 (96.2)	22 (100)	0.350
	2~4	4 (3.2)	4 (3.8)		

Histologic differentiation, n (%)	WD	16 (12.7)	10 (9.6)	6 (27.3)	0.059
	MD	75 (59.5)	65 (62.5)	10 (45.5)	
	PD	30 (23.8)	26 (25.0)	4 (18.2)	
	UE	5 (4.0)	3 (2.9)	2 (9.1)	
Tumor size, cm	Mean (±SD)	3.0 (±1.1)	3.0 (±1.0)	3.4 (±1.7)	0.076
Metastatic LN size, cm	Mean (±SD)	2.2 (±1.3)	2.3 (±1.3)	1.8 (±1.3)	0.440
cT stage, n (%)	II/III	115 (91.2)	95 (91.3)	20 (91.0)	0.947
	IV	11 (8.8)	9 (8.7)	2 (9.0)	
cStage, n (%)	II/III	33 (26.2)	26 (25.0)	7 (31.8)	0.509
	IV	93 (73.8)	78 (75.0)	15 (68.2)	
pT stage, n (%)	II/III	110 (87.3)	90 (86.5)	20 (91.0)	0.576
	IV	16 (12.7)	14 (13.5)	2 (9.0)	
pStage, n (%)	II/III	31 (24.6)	25 (24.0)	6 (27.3)	0.749
	IV	95 (75.4)	79 (76.0)	16 (72.7)	
Surgical margin, n (%)	Negative	78 (61.9)	62 (59.6)	16 (72.7)	0.250
	Positive	48 (38.1)	42 (40.4)	6 (27.3)	
ECE, n (%)	Absent	77 (61.6)	64 (61.5)	14 (63.6)	0.854
	Present	48 (38.4)	40 (38.5)	8 (36.4)	
PNI, n (%)	Absent	112 (88.9)	93 (89.4)	19 (86.4)	0.678
	Present	14 (11.1)	11 (10.6)	3 (13.6)	
LVI, n (%)	Absent	93 (73.8)	74 (71.2)	19 (86.4)	0.140
	Present	33 (26.2)	30 (28.8)	3 (13.6)	

Abbreviations: PY= pack-years; ECOG= Eastern Cooperative Oncology Group; WD= well differentiated; MD= moderately differentiated; PD= poorly differentiated; UE= unevaluable; LN= lymph node; pStage= pathological stage; ECE= extracapsular extension; PNI= perineural invasion; LVI= lymphovascular invasion; RT= radiotherapy; CRT= chemoradiotherapy

Table 2. Treatment characteristics

Variables		All, N=126	p16 expression		*p*
			p16 (+), N=104	p16 (-), N=22	
Surgical approach, n (%)	Open procedure	108 (85.7)	87 (83.7)	21 (95.5)	0.151
	Transoral robotic surgery	18 (14.3)	17 (16.3)	1 (4.5)	
Type of neck dissection, n (%)	Selective neck dissection	19 (15.1)	15 (14.4)	4 (18.2)	0.827
	Modified neck dissection	61 (48.4)	52 (50.0)	9 (40.9)	
	Radical neck dissection	45 (35.7)	36 (34.6)	9 (40.9)	
	NA[a]	1 (0.8)	1 (1.0)		
Extent of neck dissection, n (%)	Unilateral	74 (58.7)	64 (61.5)	10 (45.5)	0.314
	Bilateral	51 (40.5)	39 (37.5)	12 (54.5)	
	NA[a]	1 (0.8)	1 (1.0)		
Number of dissected neck node[b]	Median (range)	52 (10-149)	51 (12-118)	59 (10-149)	0.447
Number of metastatic neck node[b]	Median (range)	2 (0-20)	2 (0-20)	3 (0-7)	0.836
Modality of RT, n (%)	Conventional	27 (21.4)	19 (18.3)	8 (36.4)	0.060
	IMRT	99 (78.6)	85 (81.7)	14 (63.6)	
Total dose of RT, Gy	Median (range)	63 (50.4-75.9)	63 (50.4-75.9)	63 (54.0-68.4)	0.852
Fractionated dose of RT, Gy	Median (range)	1.8 (1.5-2.3)	1.8 (1.5-2.2)	1.9 (1.5-2.3)	0.120
Adjuvant therapy, n (%)	RT alone	89 (70.6)	71 (68.3)	18 (81.8)	0.205
	CRT	37 (29.4)	33 (31.7)	4 (18.2)	
Regimen of concurrent chemotherapy[c], n (%)	Cisplatin	34 (91.9)	31 (93.9)	3 (75.0)	0.179
	Cetuximab	2 (5.4)	1 (3.0)	1 (25.0)	
	TS-1	1 (2.7)	1 (3.0)		

Abbreviations: RT= radiotherapy; CRT= chemoradiotherapy; IMRT= intensity modulated radiotherapy; NA= not applicable; TS-1= oral fluoropyrimidine anticancer drug

[a] One patient refused the neck node dissection; [b] Calculation only includes patients underwent neck dissection (n=125); [c] Calculation only includes patients treated with postoperative CRT (n=40)

Table 3. Comparison of characteristics according to adjuvant therapy (N=126)

Variables		Adjuvant therapy		*p*
		RT alone, N=89	CRT, N=37	
Age, n (%)	<60 years	49 (55.1)	20 (54.1)	0.918
	≥60 years	40 (44.9)	17 (45.9)	
Smoking, n (%)	< 10 PY[a]	59 (66.3)	19 (51.4)	0.116
	≥10 PY	30 (33.7)	15 (48.6)	
Histologic differentiation, n (%)	WD	11 (12.4)	5 (13.5)	0.736
	MD	55 (61.8)	20 (54.1)	
	PD	19 (21.3)	11 (29.7)	
	UE	4 (4.5)	1 (2.7)	
p16 expression	Negative	18 (20.2)	4 (10.8)	0.205
	Positive	71 (79.8)	33 (89.2)	
Tumor size, n (%)[b]	< 3cm	38 (42.7)	18 (48.6)	0.475
	≥3cm	48 (53.9)	19 (51.4)	
Metastatic LN size, n (%)[b]	< 2cm	35 (42.7)	7 (20.0)	0.012
	≥2cm	47 (57.3)	28 (80.0)	
pT stage, n (%)	II/III	76 (85.4)	34 (91.9)	0.318
	IV	13 (14.6)	3 (8.1)	
Surgical margin, n (%)	Negative	56 (62.9)	22 (59.5)	0.716
	Positive	33 (37.1)	15 (40.5)	
ECE, n (%)	Absent	67 (75.3)	11 (29.7)	<0.001
	Present	22 (24.7)	26 (70.3)	
Multiple metastatic LN	Absent	33 (37.1)	3 (8.1)	<0.001
	Present	56 (62.9)	34 (91.9)	
PNI, n (%)	Absent	86 (96.6)	26 (70.3)	<0.001

	Present	3 (3.4)	11 (29.7)	
LVI, n (%)	Absent	77 (86.5)	16 (43.2)	<0.001
	Present	12 (13.5)	21 (56.8)	
High risk factors[c]	Absent	45 (50.6)	5 (13.5)	<0.001
	Present	44 (49.4)	32 (86.5)	
Risk factors[d]	Absent	34 (38.2)	5 (13.5)	
	Present	55 (61.8)	32 (86.5)	0.006

Abbreviations: PY= pack-years; ECOG= Eastern Cooperative Oncology Group; WD= well differentiated; MD= moderately differentiated; PD= poorly differentiated; UE= unevaluable; LN= lymph node; pStage= pathological stage; ECE= extracapsular extension; PNI= perineural invasion; LVI= lymphovascular invasion; RT= radiotherapy; CRT= chemoradiotherapy

a Calculation includes patients had smoking history that both non-smoker and below 10 PY

[b] Calculation includes only patients had pathological report

[c] Includes features such as positive surgical margin and ECE

[d] Includes features such as positive surgical margin, ECE, multiple metastatic LN, PNI, and LVI

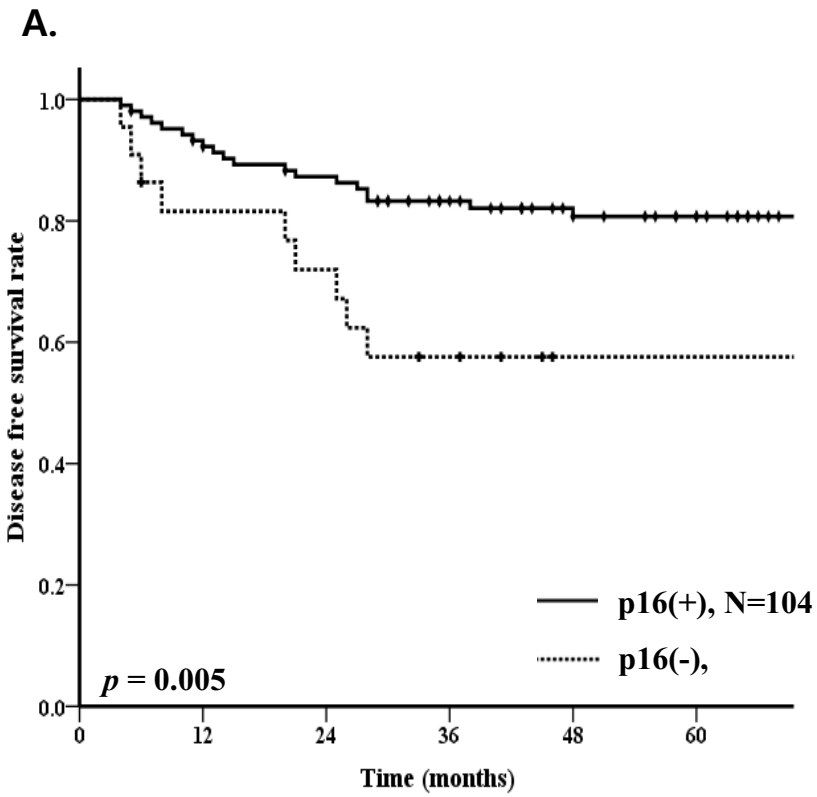
2. Outcomes and prognostic factors in entire patients

With a median follow-up of 56 months (range, 4-157 months), 20 patients

(15.9%) developed recurrences, including 9 loco-regional recurrences, 9 distant metastases, and 2 both loco-regional and distant metastases. We identified 13 recurrences (12.5%; 5 loco-regional, 6 distant metastases, and 2 both loco-regional and distant metastases) in the p16 (+) patients and 7 recurrences (31.8%; 4 loco-regional and 3 distant metastases) in the p16 (−) patients. By analyzing these recurrences, we found significantly fewer recurrences in p16 (+) patients than in p16 (-) patients (12.5% vs. 31.8%, p = 0.024). However, there was no significant difference in patterns of failure (p = 0.062) between the two groups. Furthermore, we observed that both loco-regional recurrence rate (6.7% vs. 18.2%, p = 0.084) and distant metastasis rate (7.7% vs. 13.6%, p = 0.370) between both groups were comparable. The 5-year DFS was 76.7% for the entire patient cohort. Patients with p16 (+) OPSCC exhibited a significantly better 5-year DFS than those with p16 (−) OPSCC (80.7% vs. 57.6%, p < 0.005, Figure 1A). Univariate analysis revealed other independent predictors of DFS, including pathologic T stage (p = 0.024), PNI (p = 0.007), and LVI (p = 0.028). On multivariate Cox regression model in a stepwise method, p16 (+) status (hazard ratio [HR], 0.19; 95% confidence interval [CI], 0.08-0.44, p < 0.001), pathologic T4 stage (HR, 2.67; 95% CI, 1.09-7.34, p = 0.033), and positive LVI (HR, 2.74; 95% CI, 1.25-6.00, p = 0.01) were significant prognosticators for DFS (Table 4).

Twenty-five patients died of the disease during the follow-up period. The 5-year OS in all patients was 80.4%. Patients with p16 (+) OPSCC had a significantly better 5-year OS than patients with p16 (−) OPSCC (84.9% vs. 59.1%, p < 0.001,

Figure 1B). In addition, univariate analysis demonstrated that both p16 expression status ($p < 0.001$) and PNI ($p = 0.048$) correlated significantly with OS rates, while surgical margin ($p = 0.053$) exhibited a correlative trend with OS. The stepwise multivariate analysis identified both p16 (+) expression (HR, 0.09; 95% CI, 0.03-0.23, $p < 0.001$) and positive surgical margin (HR, 2.50; 95% CI, 1.03-6.07, $p = 0.043$) as independent prognostic factors of OS (Table 4).



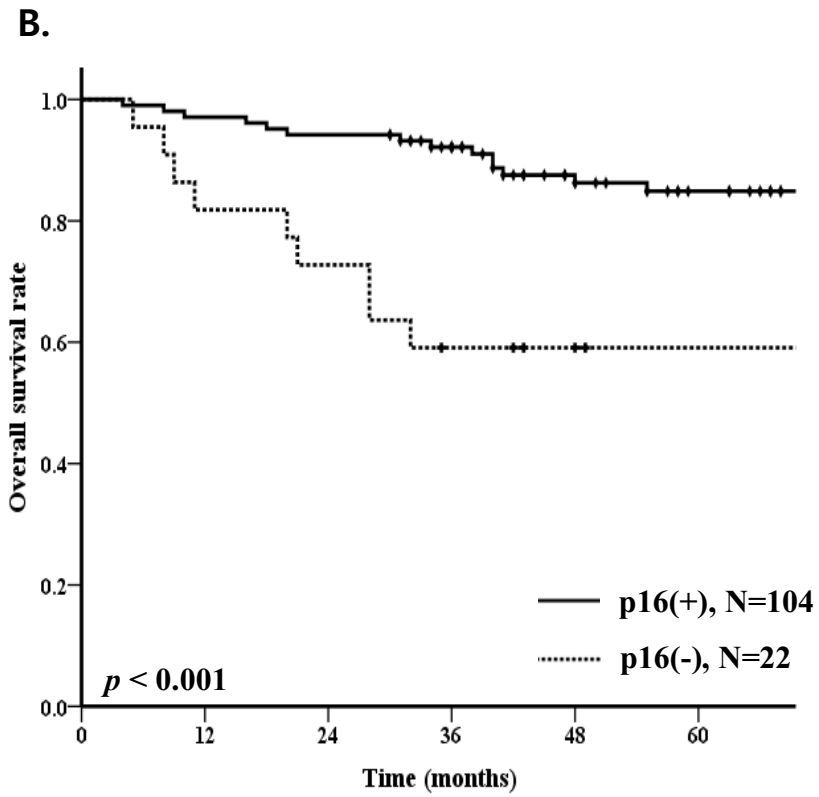


Fig. 1. Disease-free survival (A) and overall survival (B) according to p16 expression status

Table 4. Univariate and multivariate analyses of potential prognostic factors for DFS and OS

Variable		DFS				OS			
		UVA		MVA*		UVA		MVA*	
		5-Y DFS	*p*	HR (95% CI)	*p*	5-Y OS	*p*	HR (95% CI)	*p*
Age	<60	81.0	0.112	2.070 (0.996-4.304)	0.051	82.7	0.209	NI	
	≥60	70.8				77.5			
Sex	Male	74.1	0.463	NI		77.7	0.439	NI	

	Female	93.8				100.0			
Smoking	< 10 PY[a]	78.7	0.560	NI		81.2	0.979	NI	
	≥10 PY	72.8				78.4			
Histologic differentiation	WD	93.8	0.333	NI		93.8	0.509	NI	
	MD	75.9				79.3			
	PD	69.0				75.4			
p16 expression	Negative	57.6	0.005	Ref	<0.001	59.1	<0.001	Ref	<0.001
	Positive	80.7		0.186 (0.078-0.443)		84.9		0.087 (0.033-0.229)	
cT Stage	II-III	78.8	0.047	NI		81.0	0.465	NI	
	IV	54.5				72.7			
cStage	II-III	87.5	0.361	NI		89.0	0.359	NI	
	IV	72.9				77.4			
pT Stage	I-III	80.0	0.024	Ref	0.033	83.3	0.063	Ref	0.047
	IV	56.3		2.823 (1.085-7.342)		62.5		2.847 (1.012-8.007)	
pStage	I-III	92.4	0.070	Ref	0.081	92.8	0.195	NI	
	IV	71.7		2.669 (0.887-8.032)		76.5			
Surgical margin	Negative	82.5	0.161	Ref	0.091	87.9	0.053	Ref	0.043
	Positive	68.1		1.955 (0.898-4.259)		69.1		2.500 (1.029-6.069)	
ECE	Absent	82.9	0.074	NI		84.7	0.140	NI	
	Present	66.1				73.3			
PNI	Absent	80.5	0.007	NI		83.0	0.048	NI	
	Present	42.9				56.8			
LVI	Absent	82.3	0.028	Ref	0.012	84.4	0.147	NI	
	Present	60.1		2.740 (1.252-5.998)		66.9			
Adjuvant therapy	RT	80.5	0.221	NI		81.4	0.852	NI	
	CRT	65.4				74.6			

Abbreviations: DFS= disease free survival; OS= overall survival; UVA= univariate

analysis; MVA= multivariate analysis; HR= hazard ratio; CI= confidence interval; NI= not included; Ref= reference; PY= pack-years; WD= well differentiated; MD= moderately differentiated; PD= poorly differentiated; LN= lymph node; pStage= pathological stage; ECE= extracapsular extension; PNI= perineural invasion; LVI= lymphovascular invasion; RT= radiotherapy; CRT= chemoradiotherapy

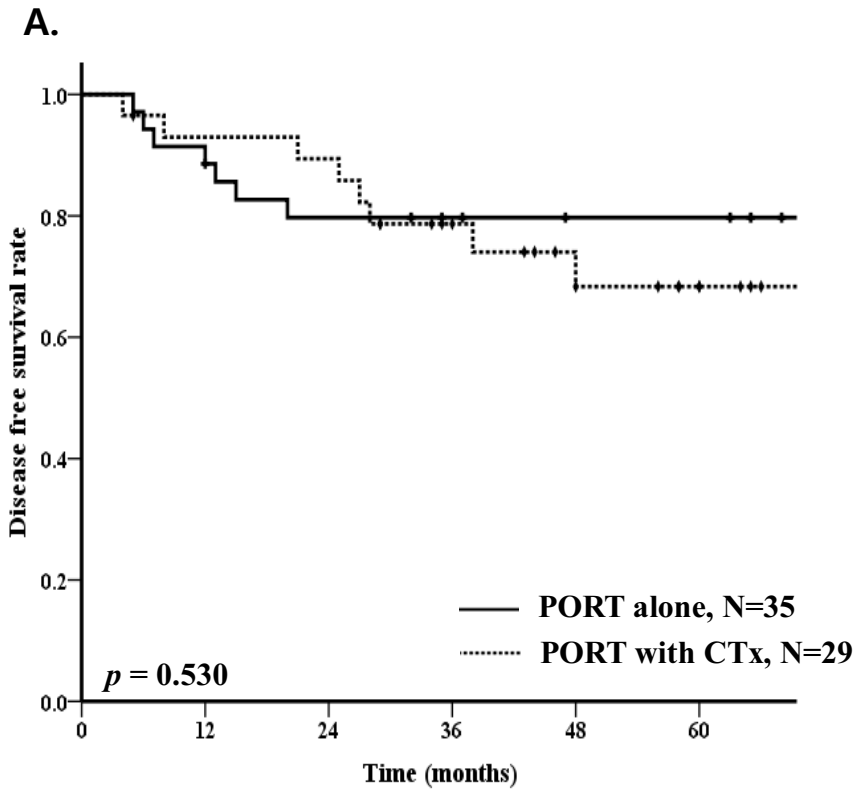
[a] Calculation includes patients had smoking history that both non-smoker and below 10 PY

*Variables were entered into the multivariate Cox regression model in a stepwise method if $P<0.10$ and were removed at any point if $P\geq0.10$

3. Outcomes in p16 (+) OPSCC patients with high risk factors

To gain insights into optimized adjuvant therapy, we analyzed 64 patients with p16 (+) OPSCC with high risk factors. The 5-year DFS and OS were 75.1% and 80.7%, respectively. In terms of adjuvant therapy (PORT alone vs. PORT with adjuvant chemotherapy), the full details of patients and their tumor characteristics treated with both treatments are listed in the Table 5. The 5-year DFS did not differ significantly for patients who received PORT alone (n = 35) compared to those who received PORT with adjuvant chemotherapy (n = 29) (79.7% vs. 68.3%; p = 0.531) (Figure 2A). Similarly, there were no significant differences in 5-year OS for patients who received PORT alone versus PORT with adjuvant chemotherapy (82.1% vs.

76.2%; p = 0.964) (Figure 2B). The modality of adjuvant treatment (PORT vs. PORT with chemotherapy) did not significantly affect either DFS or OS in a multivariate Cox regression model in a stepwise method. Only LVI was identified as an independent risk factor for DFS (HR, 6.41; 95% CI, 1.27-32.41, p = 0.025) and OS (HR, 3.26; 95% CI, 1.14-9.30, p = 0.027). Data on multivariate analyses to assess potential prognostic factors for survival in p16 (+) OPSCC patients with high risk factors are shown in Table 6.



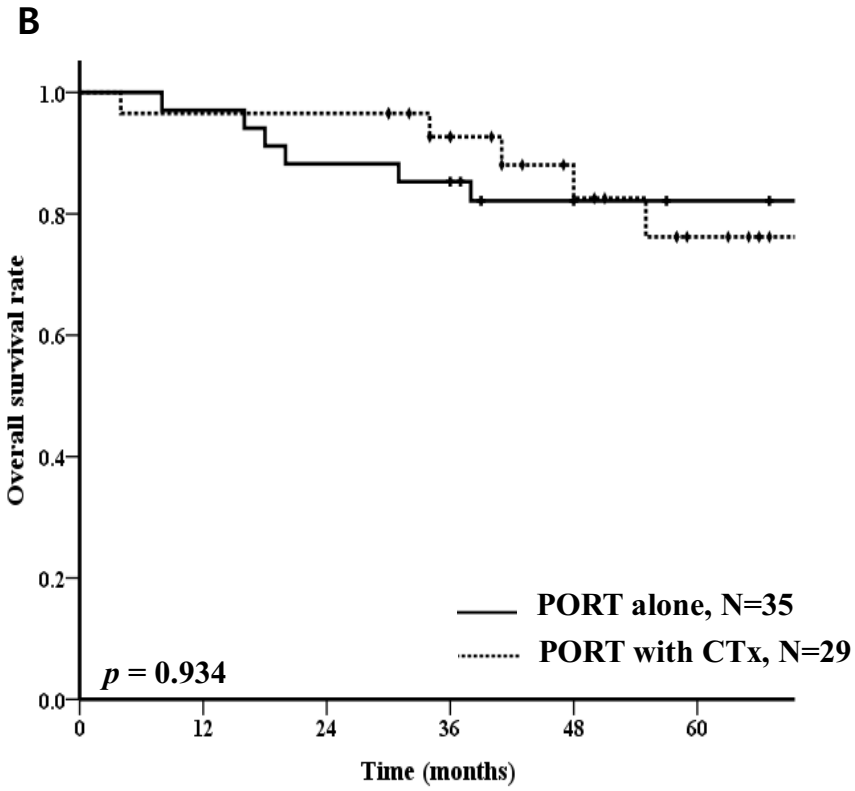


Fig. 2. Disease-free survival (A) and overall survival (B) in p16 (+) OPSCC patients with high risk factors that were treated with postoperative radiotherapy (PORT) alone versus PORT with chemotherapy (CTx)

Table 5. Baseline characteristics according to adjuvant therapy in p16 (+) OPSCC patients with high risk factors (N=64)

Variables		Adjuvant therapy		*p*
		RT alone, N=35	CRT, N=29	
Age, n (%)	<60 years	19 (54.3)	16 (55.2)	0.943
	≥60 years	16 (45.7)	13 (44.8)	
Smoking, n (%)	< 10 PY[a]	22 (62.9)	15 (51.7)	0.369
	≥10 PY	13 (37.1)	14 (48.3)	
Histologic differentiation, n (%)	WD	2 (5.7)	4 (13.8)	0.667

	MD	21 (60.0)	14 (48.3)	
	PD	11 (31.4)	10 (34.5)	
	UE	1 (2.9)	1 (3.4)	
Tumor size, n (%)[b]	< 3cm	18 (51.4)	15 (51.7)	0.651
	≥3cm	17 (48.6)	14 (48.3)	
Metastatic LN size, n (%)[b]	< 2cm	9 (27.3)	5 (17.9)	0.400
	≥2cm	24 (72.7)	22 (78.6)	
pT stage, n (%)	II/III	29 (82.9)	26 (89.7)	0.436
	IV	6 (17.1)	3 (10.3)	
Surgical margin, n (%)	Negative	8 (22.9)	14 (48.3)	0.033
	Positive	27 (77.1)	15 (51.7)	
ECE, n (%)	Absent	18 (51.4)	6 (20.7)	0.011
	Present	17 (48.6)	23 (79.3)	
Multiple metastatic LN	Absent	9 (25.7)	3 (10.3)	0.117
	Present	26 (74.3)	26 (89.7)	
PNI, n (%)	Absent	34 (97.1)	20 (69.0)	0.002
	Present	1 (2.9)	9 (31.0)	
LVI, n (%)	Absent	29 (82.9)	11 (37.9)	< 0.001
	Present	6 (17.1)	18 (62.1)	

Abbreviations: RT= radiotherapy; CRT= chemoradiotherapy; PY= pack-years; ECOG= Eastern Cooperative Oncology Group; WD= well differentiated; MD= moderately differentiated; PD= poorly differentiated; UE= unevaluable; LN= lymph node; pStage= pathological stage; ECE= extracapsular extension; PNI= perineural invasion; LVI= lymphovascular invasion

[a] Calculation includes patients had smoking history that both non-smoker and below 10 PY

[b] Calculation includes only patients had pathological report

Table 6. Multivariate analyses of potential prognostic factors for DFS and OS in p16 (+) OPSCC patients with high risk factors (N=64)

Variable		MVA*			
		DFS		OS	
		HR (95% CI)	*p*	HR (95% CI)	*p*
Surgical margin, n (%)	Negative	NI		Ref	0.060
	Positive			3.461 (0.950-12.612)	
ECE, n (%)	Absent	NI		NI	
	Present				
Multiple metastatic LN	Absent	Ref	0.150	Ref	0.127
	Present	4.968 (0.559-44.156)		4.929 (0.637-38.160)	
PNI, n (%)	Absent	NI		NI	
	Present				
LVI, n (%)	Absent	Ref	0.025	Ref	0.027
	Present	6.405 (1.266-32.410)		3.258 (1.141-9.300)	
Adjuvant therapy	RT	NI		NI	
	CRT				

Abbreviations: DFS= disease free survival; OS= overall survival; MVA= multivariate analysis; HR= hazard ratio; CI= confidence interval; NI= not included; Ref= reference; ECE= extracapsular extension; PNI= perineural invasion; LVI= lymphovascular invasion; RT= radiotherapy; CRT= chemoradiotherapy

*Variables were entered into the multivariate Cox regression model in a stepwise method if $P<0.10$ and were removed at any point if $P \geq 0.10$

IV. DISCUSSION

This study showed the clinical significance of p16 expression in a retrospective, unselected cohort of 126 patients with OPSCC. In the period 2000–2011, p16 positivity in our cohorts was 82.5%. This prevalence rate is comparable to that in the United States and Europe [1,2,8]. Additionally, a meta-analysis suggests that the proportion of OPSCC associated with HPV has increased from 40.5% before the year 2000 to 72.2% after 2005 [2]. We reported that patients with p16 (+) OPSCC, managed with surgery followed by PORT with or without chemotherapy, showed significantly better 5-year DFS (80.7% vs. 57.6%, $p < 0.001$) and OS survival (84.9% vs. 59.1%, $p < 0.001$) than those with p16 (-) tumors. This could be understood in the same context that, in the literature, HPV infection and/or p16 positivity are associated with improved survival in OPSCC patients treated with primary RT and CRT [5,6,9]. Indeed, p16 (+) OPSCC are a distinct type of cancer with a generally better outcome than p16 (−) disease, which may be independent of the treatment modality chosen.

The standard of care of OPSCC is multimodality therapy based on several factors, including clinical stage, individual patient factors such as comorbidities and preferences, and particularly, the institutional preference based on clinical discretion of the physician. Recently data suggest that most institutions prefer primary RT/CRT to surgery followed by PORT, as the former helps to preserve the organ in advanced OPSCC patients. Consequently, the majority of publications have focused on patients with OPSCC that received definitive RT. Generally, these conclusions regarding

prognosis in HPV-positive OPSCC were based on results from tumors treated with definitive CRT [5-7,9]. The prognosis of HPV-positive OPSCC after surgical resection and adjuvant PORT is relatively unclear because of a small number of clinical studies, although this might be presumed good prognosis. Previous literature implied that HPV positivity is a predictor of prognosis for OPSCC in the postoperative setting. Haughey et al. reported that 171 p16 (+) OPSCC patients treated with transoral laser microsurgery had excellent survival outcomes including 5-year OS, disease-specific survival (DSS), and DFS of 91%, 94%, 88%, respectively [10]. Rahmati et al. reported that patients who were p16 (+) had superior OS and DSS compared with patients who were p16 (-) (5-year OS, 74% vs. 47%; p = 0.04 and 5-year DSS, 89% vs. 66%; p = 0.08) [11]. In our institution, surgery followed by RT was the mainstay for treating patients with OPSCC, based on clinical factors and physician discretion, including concern for the short/long-term toxicity of RT. Recently, surgical management of OPSCC has seen increasing application with advances in minimally invasive surgery, such as robotic and transoral laser microsurgery. So, we assessed the prognosis of OPSCC managed with surgery followed by PORT stratified by p16 expression status. We confirmed that p16 (+) expression in OPSCC is an independent and favorable prognostic factor related to DFS and OS in these patients regardless of whether they received chemotherapy.

Currently, patients with HPV-positive OPSCC are treated similarly to age- and stage-matched HPV-negative counterparts, although HPV testing of OPSCC is recommended for prognostic purposes. However, treatment goals and selection of

therapy are debatable in these patients because HPV-positive OPSCC has a superior prognosis and a distinct patient profile, including younger age and good performance status [12]. In other words, because patients with HPV-positive OPSCC are expected to live longer after treatment, avoiding late toxicity and maintaining quality of life (QOL) are particularly important. Accordingly, de-intensification of therapy may be appropriate for these HPV-positive OPSCC with good prognosis to improve associated morbidity and QOL [7,13].

Treatment strategies, including PORT and adjuvant chemotherapy, for HPV positive OPSCC patients who have undergone surgical resection have not yet emerged. Traditionally, PORT has been the standard adjuvant approach postoperatively for OPSCC patients with risk factors such as positive surgical margin, ECE, multiple positive LN, PNI, and LVI. Thereafter, two major phase III randomized trials including RTOG 9501 [14] and European Organization for Research and Treatment of Cancer (EORTC) 22931 [15] identified that high-risk patients with positive surgical margins and/or ECE in lymph node metastasis had benefit from the addition of cisplatin. However, these two randomized trials did not consider the significance of HPV status. Our cohort showed a 20 – 30% increase in the 5-year DFS and OS over those of RTOG 9501 and EORTC 22931 trials. These outcomes came from a high percentage of p16 (+) disease in this cohort. The sharp increase in the proportion of HPV-positive OPSCC occurred after the year 2000, and the percentage of carcinogenic HPV in the etiology of OPSCC has doubled over the last decade [2]. The cohorts of RTOG 9501 and EORTC 22931 were treated with PORT or PORT with

chemotherapy before 2000, while the cohort in this study was treated after the year 2000.

We observed that patients with p16 (+) OPSCC and high-risk factors (n=63) had excellent DFS and OS of 75.1% and 80.7%, respectively, although only 45% of these patients received PORT with chemotherapy. It is reasonable to propose that de-intensification adjuvant treatment may be considered in the management of select p16 (+) OPSCC with high risk features showed favorable outcome regardless of concurrently adjuvant chemotherapy. Based on our findings, a re-evaluation of the routine application of concurrent chemotherapy during PORT for p16 (+) OPSCC with high risk factors may be warranted. Also, in this study, it is noteworthy that LVI, which is traditionally considered a minor or moderate risk factor, was a more important risk factor for survival than ECE in p16 (+) OPSCC. Maxwell reported that ECE, a long-established major risk factor, was not significantly associated with worse disease-specific survival in p16-positive OPSCC patients [16]. Ultimately, the superior prognosis associated with p16 (+) disease may indicate a need to re-examine traditional risk factors and stratification in the postoperative setting.

Despite the overall good prognosis for HPV-positive OPSCC, some aggressive subtypes have been described, characterized by distant spread [17] and advanced nodal stage [18]. Likewise, some patients with HPV-positive OPSCC remain at risk of poor outcome, complicating de-intensification efforts. Therefore, we should classify risk group for studies testing de-intensification approaches. Currently, de-intensification trials are being conducted for HPV-positive OPSCC based on risk

factors in the postoperative setting. The phase III ADEPT (NCT01687413) trial [19] is investigating a treatment de-intensification strategy by comparing RT alone to CRT in HPV-positive OPSCC patients with ECE in lymph node metastasis and a negative surgical margin who also underwent surgery. Also, the Eastern Cooperative Oncology Group (ECOG) 3311 (NCT01898494) [20] is conducting a phase II trial in which patients with resectable p16 (+) OPSCC are stratified into 4-arm treatments according to their surgical pathology after transoral surgery. In that study, patients are randomized into either low-dose or standard-dose PORT, with or without chemotherapy.

Our study has some limitations. Owing to data with retrospective in nature, we could not assess functional outcomes of swallowing, salivation, speech, and diet. In addition, it was comprised of relatively small patient cohorts. Next, the use of p16 immunochemistry as a sole marker for HPV positivity is unsatisfactory. Although p16 overexpression is a sensitive technique to detect the presence of HPV in OPSCC, polymerase chain reaction testing and in situ hybridization would further improve the validation [21,22]. Finally, our data did not examine an association between p16 expression and molecular biomarkers such as epidermal growth factor receptor and p53 in OPSCC, which could provide important prognostic information [23-25].

V. CONCLUSION

HPV positivity based on expression of p16 is a strong and independent prognosticator of survival in OPSCC treated with surgical resection followed by PORT. Future research will confirm whether the traditional risk factors and risk stratification applies equally to the HPV positive cohort. Additional studies will be able to validate optimal de-intensification approaches according to the risk group for p16 (+) OPSCC in the postoperative setting.

REFERENCES

1. Chaturvedi AK, Engels EA, Anderson WF, Gillison ML. Incidence trends for human papillomavirus-related and -unrelated oral squamous cell carcinomas in the United States. J Clin Oncol 2008;26:612-9.
2. Mehanna H, Beech T, Nicholson T, El-Hariry I, McConkey C, Paleri V, et al. Prevalence of human papillomavirus in oropharyngeal and nonoropharyngeal head and neck cancer--systematic review and meta-analysis of trends by time and region. Head Neck 2013;35:747-55.
3. Dahlstrom KR, Calzada G, Hanby JD, Garden AS, Glisson BS, Li G, et al. An evolution in demographics, treatment, and outcomes of oropharyngeal cancer at a major cancer center: a staging system in need of repair. Cancer 2013;119:81-9.
4. Kreimer AR, Clifford GM, Boyle P, Franceschi S. Human papillomavirus types in head and neck squamous cell carcinomas worldwide: a systematic review. Cancer Epidemiol Biomarkers Prev 2005;14:467-75.
5. D'Souza G, Kreimer AR, Viscidi R, Pawlita M, Fakhry C, Koch WM, et al. Case-control study of human papillomavirus and oropharyngeal cancer. N Engl J Med 2007;356:1944-56.
6. Ang KK, Harris J, Wheeler R, Weber R, Rosenthal DI, Nguyen-Tan PF, et al. Human papillomavirus and survival of patients with oropharyngeal cancer. N Engl J Med 2010;363:24-35.
7. O'Sullivan B, Huang SH, Siu LL, Waldron J, Zhao H, Perez-Ordonez B, et al.

Deintensification candidate subgroups in human papillomavirus-related oropharyngeal cancer according to minimal risk of distant metastasis. J Clin Oncol 2013;31:543-50.

8. Marur S, D'Souza G, Westra WH, Forastiere AA. HPV-associated head and neck cancer: a virus-related cancer epidemic. Lancet Oncol 2010;11:781-9.
9. Rischin D, Young RJ, Fisher R, Fox SB, Le QT, Peters LJ, et al. Prognostic significance of p16INK4A and human papillomavirus in patients with oropharyngeal cancer treated on TROG 02.02 phase III trial. J Clin Oncol 2010;28:4142-8.
10. Haughey BH, Sinha P. Prognostic factors and survival unique to surgically treated p16+ oropharyngeal cancer. Laryngoscope 2012;122 Suppl 2:S13-33.
11. Rahmati R, Dogan S, Pyke O, Palmer F, Awad M, Lee N, et al. Squamous cell carcinoma of the tonsil managed by conventional surgery and postoperative radiation. Head Neck 2014; doi:10.1002/hed.23679.
12. D'Souza G, Zhang HH, D'Souza WD, Meyer RR, Gillison ML. Moderate predictive value of demographic and behavioral characteristics for a diagnosis of HPV16-positive and HPV16-negative head and neck cancer. Oral Oncol 2010;46:100-4.
13. Masterson L, Moualed D, Liu ZW, Howard JE, Dwivedi RC, Tysome JR, et al. De-escalation treatment protocols for human papillomavirus-associated oropharyngeal squamous cell carcinoma: A systematic review and meta-analysis of current clinical trials. Eur J Cancer 2014; doi:10.1016/j.ejca.2014.07.001.

14. Cooper JS, Pajak TF, Forastiere AA, Jacobs J, Campbell BH, Saxman SB, et al. Postoperative concurrent radiotherapy and chemotherapy for high-risk squamous-cell carcinoma of the head and neck. N Engl J Med 2004;350:1937-44.
15. Bernier J, Domenge C, Ozsahin M, Matuszewska K, Lefebvre JL, Greiner RH, et al. Postoperative irradiation with or without concomitant chemotherapy for locally advanced head and neck cancer. N Engl J Med 2004;350:1945-52.
16. Maxwell JH, Ferris RL, Gooding W, Cunningham D, Mehta V, Kim S, et al. Extracapsular spread in head and neck carcinoma: impact of site and human papillomavirus status. Cancer 2013;119:3302-8.
17. Huang SH, Perez-Ordonez B, Weinreb I, Hope A, Massey C, Waldron JN, et al. Natural course of distant metastases following radiotherapy or chemoradiotherapy in HPV-related oropharyngeal cancer. Oral Oncol 2013;49:79-85.
18. McIlwain WR, Sood AJ, Nguyen SA, Day TA. Initial symptoms in patients with HPV-positive and HPV-negative oropharyngeal cancer. JAMA Otolaryngol Head Neck Surg 2014;140:441-7.
19. Washington University School of Medicine. Post operative adjuvant therapy de-intensification trial for human papillomavirus-related, p16+ oropharynx cancer (ADEPT). Available at http://clinicaltrials.gov/ct2/show/NCT01687413?term=NCT01687413&rank=1 [Accessed Aug 28 2014]
20. Eastern Cooperative Oncology Group. Transoral surgery followed by low-dose

or standard-dose radiation therapy with or without chemotherapy in treating patients with HPV positive stage III-IVA oropharyngeal cancer. Available at http://clinicaltrials.gov/ct2/show/NCT01898494?term=NCT01898494&rank=1 [Accessed Aug 28 2014]

21. Ukpo OC, Flanagan JJ, Ma XJ, Luo Y, Thorstad WL, Lewis JS, Jr. High-risk human papillomavirus E6/E7 mRNA detection by a novel in situ hybridization assay strongly correlates with p16 expression and patient outcomes in oropharyngeal squamous cell carcinoma. Am J Surg Pathol 2011;35:1343-50.
22. Lewis JS, Jr. p16 Immunohistochemistry as a standalone test for risk stratification in oropharyngeal squamous cell carcinoma. Head Neck Pathol 2012;6 Suppl 1:S75-82.
23. Kozomara R, Jovic N, Magic Z, Brankovic-Magic M, Minic V. p53 mutations and human papillomavirus infection in oral squamous cell carcinomas: correlation with overall survival. J Craniomaxillofac Surg 2005;33:342-8.
24. Fallai C, Perrone F, Licitra L, Pilotti S, Locati L, Bossi P, et al. Oropharyngeal squamous cell carcinoma treated with radiotherapy or radiochemotherapy: prognostic role of TP53 and HPV status. Int J Radiat Oncol Biol Phys 2009;75:1053-9.
25. Reimers N, Kasper HU, Weissenborn SJ, Stutzer H, Preuss SF, Hoffmann TK, et al. Combined analysis of HPV-DNA, p16 and EGFR expression to predict prognosis in oropharyngeal cancer. Int J Cancer 2007;120:1731-8.

ABSTRACT (IN KOREAN)

수술적 치료를 받은 구인두암 환자에서 인체유두종바이러스 감염이 미치는 영향: 전통적인 위험 계층화에 대한 재평가의 필요성

<지도교수 금 기 창>

연세대학교 대학원 의학과

이 정 심

목적: 본 연구에서는 국소진행성 구인두암 환자에서 수술과 수술후방사선의 병합 치료를 시행하였을 때, p16 상태가 치료성적에 미치는 영향을 알아보고자 하였다. 더불어 p16 양성인 구인두암 환자에 대한 수술 후 치료 조정(modulation)의 필요성에 대해 조사해 보고자 한다.

대상 및 방법: 2001년 1월에서 2011년 12월 사이에 구인두암으로 진단 받고 수술과 수술후방사선의 병합 치료시행 받았으며, p16 상태를 알 수 있는 126명의 환자를 대상으로 진행되었다. 모든 환자들은 수술을 시행 받은 후 수술 위험 계층화 중 고위험군 (양성 수술 절제연, 피막 외 신장) 이나 다른 위험 인자를 가지고 있는 위험군 (다발 임파선 전이, 임파주위 혈관주위 침범)에 해당하는 경우로 수술 후 방사선치료 ± 항암치료를 시행하였다. 환자들이

조사받은 수술 후 방사선 치료 선량의 중앙값은 63.0 Gy (범위, 50.4-78.6 Gy) 이었다.

결과: 대상환자 중 104명 (82.5%) 의 환자는 p16 양성 (p16 (+))이었고, 22명 (17.5%) 환자는 p16 음성(p16 (-)) 이었다. 환자의 중앙추적조사기간은 56 개월 (범위, 4-157 개월) 이었다. 전체 환자의 5년 무병생존율과 생존율은 각각 76.7%, 80.4% 이었다. p16 (+)인 환자군이 p16 (-) 환자군에 비해 5년 무병생존율 (80.7% vs. 57.6%, $p < 0.001$)과 생존율(84.9% vs. 59.1%, $p < 0.001$)이 모두 통계학적으로 유의하게 높았다. p16 (+)인 환자 중 전통적인 두경암 환자에서 수술 후 고위험군(양성 수술 절제연, 피막 외 신장)에 해당하는 64명의 환자를 대상으로 수술 후 방사선치료와 수술 후 항암방사선치료의 치료방법에 따른 5년 무병생존율 (80.7% vs. 57.6%, $p < 0.001$)과 생존율(84.9% vs. 59.1%, $p < 0.001$)를 비교하였을 때 차이를 보이지 않았다.

결론: 국소진행성 구인두암 환자에 대해 수술과 수술 후 방사선의 병합 치료를 시행하였을 때, p16 상태는 생존율에 영향을 주는 인자로 확인되었다. p16 (+) 구인두암의 양호한 예후는 현재 적용되고 있는 전통적인 수술 후 위험 계층화를 재검토하여 p16 (+) 구인두암에 대한 최적의 수술 후 치료를 결정할 필요성이 있음을 보여준다.

핵심 되는 말: 구인두암, 인체유두종바이러스, p16, 위험 계층화