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Human Papillomavirus Infections are Common and Predict Mortality in a Retrospective Cohort Study of Taiwanese Patients With Oral Cavity Cancer

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Abstract: Human papillomavirus (HPV) infections are deemed to play a role in the pathogenesis of oral cavity cancer (OCC). However, their exact prevalence and clinical significance remain unclear. Herein, we investigated the prevalence and prognostic value of HPV infections in a large sample of Taiwanese OCC patients.

This study was designed as a retrospective cohort study. Between 2004 and 2011, we identified 1002 consecutive patients with newly diagnosed OCC who were scheduled for standard treatment. HPV genotyping was performed in tumor specimens using polymerase chain reaction-based HPV blots. To investigate the temporal trends of HPV infections and their impact on 5-year overall survival (OS), patients were divided into 2 cohorts according to calendar periods: "2004 cohort" (2004–2007; n = 466) and "2008 cohort" (2008–2011; n = 536). Univariate and multivariate Cox regression models were also used to identify the independent predictors of OS in the 2 cohorts. A weighted risk score was assigned to each factor based on the range of their corresponding hazard ratios and validated in both cohorts using the c-statistic.

The overall prevalence of HPV infections was 19%, with a trend toward decreasing rates from 2004 to 2011. In patients without risky oral habits, the 5-year OS rate of HPV-positive patients was significantly lower than that of HPV-negative cases (49% vs 80%; $P = 0.021$). In the 2004 cohort, multivariate analysis identified HPV16, pathological T3/T4, pathological N1/N2, and extracapsular spread as independent adverse prognostic factors for OS. In the 2008 cohort, pathological N1/N2, pathological stage III/IV, and histological tumor depth >8 mm were identified as independent adverse prognostic factors. Using a weighted grading system incorporating HPV16 infection, we devised a prognostic index that identified 4 distinct risk categories with 5-year OS rates ranging from 25% to 89% (c-statistic = 0.76) in the 2004 cohort. The validity of the index was internally confirmed in the 2008 cohort (c-statistic = 0.71).

We conclude that HPV infections are common in Taiwanese OCC patients and predict 5-year OS. If independently validated, our composite prognostic score comprising HPV16 infection may be useful for allocating OCC patients to risk-adapted therapies.

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Abbreviations: AJCC = American Joint Committee on Cancer, CI = confidence interval, ECS = extracapsular spread, HPV = human papillomavirus, HR = hazard ratio, IQR = interquartile range, OCC = oral cavity cancer, OPC = oropharyngeal cancer, OS = overall survival.

INTRODUCTION

Oral cavity cancer (OCC) and oropharyngeal cancer (OPC) are among the most common cancers worldwide, with an estimated 440,000 new cases occurring in 2012.[1] Despite being an established cause of OPC (>50%), molecular epidemiology data demonstrated that the risk of OCC attributable to human papillomavirus (HPV) infections is relatively low (~3%).[2,3] Notably, the incidence of OPC has overall increased slightly over time, although not significantly so. Such an increase was largely driven by HPV-related OPC from 1983 to 2002, whereas OCC concomitantly showed a decreasing trend.[4,5] In general, the role of HPV infections in the pathogenesis of OCC seems to be less prominent in industrialized countries.[6]

Despite the reduction in risky oral habits (ie, betel chewing, tobacco smoking, and alcohol drinking) observed between 1980 and 2010, the incidence of OCC in Taiwan is still increasing.[7–9] Notably, HPV infections seem to be common in Asian OCC patients, being detectable in 20% to 50% of all cases.[6,10–16] Moreover, there is evidence to suggest that HPV infections may predict distant metastases,[12,16] second primary cancers,[13] disease-free survival,[14,16] disease-specific survival,[12,14–16] and overall survival (OS).[12,15,16] Unfortunately,

previous studies on the role of HPV in OCC were limited by small sample sizes and did not provide sufficient evidence to modify current clinical guidelines and management protocols. Importantly, a better understanding of the role played by HPV infections in OCC is paramount to assess whether or not an HPV vaccine may be useful for these patients.

The aims of this retrospective study were to investigate the prevalence of intratumor HPV infections in formalin-fixed paraffin-embedded primary tumor samples from a large cohort of OCC patients, and to assess the independent prognostic significance of HPV infections for 5-year OS after treatment with radical surgery(either with or without adjuvant therapy).

MATERIALS AND METHODS

Ethics Statement

The local Institutional Review Board at Chang Gung Memorial Hospital approved the study (No. 99-0112B), which followed the tenets of the Helsinki Declaration. Written informed consent was obtained from each patient.

Participants

This study was designed as a retrospective research. In total, 1002consecutive Taiwanese OCC patients were enrolled between 2004 and 2011. The inclusion criteria were as follows: newly histology-proven OCC; previously untreated tumor scheduled for radical surgery (either with or without neck dissection); absence of suspected distant metastases detected by imaging; and willingness to undergo imaging-guided biopsy or exploratory surgery if necessary. The exclusion criteria included the refusal or inability to undergo radical surgery of OCC.

Procedures

All participants underwent an extensive presurgical evaluation.[12,15] Cancer staging was performed according to the 2002 American Joint Committee on Cancer (AJCC) 6th edition staging criteria.[17] All patients underwent radical excision of the primary tumor with ≥1 cm gross safety margins. Patients with advanced-stage cancer and/or close margins ≤4 mm underwent adjuvant radiotherapy or concomitant chemoradiotherapy, as reported elsewhere.[12] The following variables were collected in all participants: sex, age at disease onset, alcohol drinking (ever/never), betel quid chewing (ever/never), cigarette smoking (ever/never), tumor subsite, differentiation, pathological T-status, pathological N-status, pathological stage, extracapsular spread (ECS), level IV/V metastases, treatment modality, and patient status at the last follow-up.

HPV Typing

Formalin-fixed, paraffin-embedded tumor samples collected during radical surgery were prepared for DNA extraction as described elsewhere.[12] DNA was extracted using a Lab Turbo 48 automatic nucleic acid extraction system and a Lab Turbo Virus Mini Kit LVN500 (Taigen, Taipei, Taiwan). We utilized a commercially available HPV L1 gene polymerase chain reaction assay (EasyChip HPV Blot genotyping assay, King Car Ltd, Ilan, Taiwan) to screen for HPV infections. The assay underwent strict quality-check procedures,[18,19] and it has been successfully utilized in previous OCC studies.[11–13,15,16] A 192-bp DNA fragment was produced after amplification of HPV DNA using the MY11/biotinylated GP6+ L1 consensus primer system. Samples that were β-globin-negative were repeatedly

extracted. Briefly, the resultant amplimers (15 μL) were hybridized to a nylon membrane that was coated with 39 HPV type-specific oligonucleotide probes.

Definitions of HPV Infections

The prevalence of genotype-specific HPV infections was reported both individually and in distinct groups (all types, oncogenic types, and nononcogenic types). The category "any HPV infection" was defined as a positive test result for 1 (single) or more than one (multiple) of the 39 distinct HPV genotypes included in the assay. Patients with both oncogenic and nononcogenic HPV types were classified as having an "oncogenic HPV infection". Because more than half of oncogenic HPV infections are caused by HPV16,[6,10–14] oncogenic HPV infections were further divided into 2 subgroups: "HPV16 infections" and "other oncogenic HPV infections".

Statistical Analysis

The main endpoint was OS (defined as the time period between primary surgery and death by any cause). Follow-up visits were continued until April 30, 2014. Patients without a documented event were censored at time of last follow-up. To investigate the temporal trends of HPV infections and their impact on 5-year OS, patients were divided into 2 cohorts according to calendar periods: "2004 cohort" (2004–2007; n = 466) and "2008 cohort" (2008–2011; n = 536). Continuous variables were categorized using published thresholds, laboratory norms, and quartiles. Data were compared using the Mann–Whitney U test and the chi-square test.

A prognostic scoring system was devised based on clinicopathological factors identified as significant for OS by multivariate analysis in the 2004 cohort. Internal validity was examined using the 2008 cohort. Survival curves were plotted using the Kaplan–Meier method and compared with the log-rank test. We used Cox proportional-hazard regression analysis with a bootstrap approach (200 runs) to identify the variables associated with survival and to estimate unadjusted and adjusted hazard ratios (HRs) and their corresponding 95% confidence intervals (95% CIs). Dichotomized variables were considered for further analysis only when they were of prognostic significance in univariate analysis. Correlations between variables were assessed with the Spearman correlation. All variables that showed significant association with OS on univariate analysis and variables of interest were consequently included in multivariate stepwise Cox proportional-hazard regression models were fitted using a backward selection procedure, with a significance level for retention of any given variable into the model of 0.05. Independent factors associated with OS in the final model were included in the prognostic index.

We assigned a weighted risk score to each factor based on the range of their corresponding HRs. The total risk score was then calculated by the sum of the ratings of individual factors. The discrimination ability of the developed models was evaluated using the Harrell c-statistic. Models are typically considered acceptable when c-values are between 0.7 and 0.8, excellent when between0.8 and 0.9, and outstanding when ≥0.9.[20] All calculations were performed using the SPSS 23.0 statistical package for Windows (SPSS Inc., Chicago, IL), the R software (Version 3.2.2; R Foundation for Statistical Computing, http://www.r-project.org/), and GraphPad Prism 6.0 for Windows (GraphPad Software, Inc., San Diego, CA). Two-tailed P values <0.05 were considered statistically significant.

RESULTS

Patient Characteristics and Clinical Outcomes

Sixty-four females and 938 males who were scheduled to receive primary surgery at our hospital were enrolled. In Taiwan, the incidence of OCC has been found to be markedly lower in females than in males ($2.23/10^6$ vs $23.67/10^6$, respectively [2004]; $2.71/10^6$ vs $25.52/10^6$, respectively [2011]).[8,9] The discrepancy of tobacco and betel quid use may account for the different incidence of OCC between male Taiwanese and female Taiwanese.[21] On the basis of the observed sex-related differences in the incidence of OCC in our country, it is not surprising that males outnumbered females in the current study. Table 1 depicts the general characteristics of the study participants. There were 723 (72%) alive patients who completed a minimum follow-up of 5 months (median: 59 mos, interquartile range [IQR]: 37–83 mos). A total of 279 (28%) patients died during the study period (median time to death: 15 mos, IQR: 9–30 mos). The 5-year OS rate in the entire study cohort was 72% (95% CI 70%–75%).

Prevalence and Temporal Trends of HPV Infections

The overall prevalence of any HPV infection in the study cohort was 19%. Notably, 2% of the participants had multiple HPV infections. No statistically significant differences in terms of baseline variables were found between HPV-positive (any HPV infection) and HPV-negative patients (Table 1). More individuals were infected with oncogenic HPV types (16%) than nononcogenic types (4%). The 3 most common genotypes (including single and multiple infections) were HPV16 (8%), HPV18 (5%), and HPV52 (2%) (Table 2). The prevalence of HPV infections in OCC patients showed a decreasing trend across calendar-years for all assays (Fig. 1). We observed a significantly lower prevalence of HPV infections in the 2008 cohort compared with the 2004 cohort (16% vs 23%; $P = 0.011$). Notably, oncogenic HPV infections were significantly less frequent (12% vs 21%; $P < 0.001$), whereas non-oncogenic HPV types were significantly more common (5% vs 2%; $P = 0.022$). Similar decreases were observed for the prevalence of both HPV16 (6% vs 10%; $P = 0.022$) and HPV18 (3% vs 7%; $P = 0.007$) infections.

Impact of HPV Infections on Survival Rates

The 5-year OS rate was 68% in HPV-positive patients ($n = 194$) and 73% in HPV-negative patients ($n = 808$; $P = 0.243$). Moreover, OCC patients with HPV16 infections ($n = 80$; 5-year OS, 67%), other oncogenic HPV infections ($n = 78$; 5-year OS, 78%), or nononcogenic HPV infections ($n = 36$; 5-year OS, 81%) did not significantly differ from those without HPV infections in terms of OS ($P = 0.341$).

Prognostic Impact of HPV Infections According to Risky Oral Habits

We then analyzed the prognostic impact of HPV infections according to the presence of risky oral habits (alcohol drinking, betel quid chewing, or cigarette smoking). The study cohort was divided according to the presence or absence of risky oral habits. Interestingly, patients without risky oral habits and HPV infections ($n = 12$) showed a worse OS (49% vs 80%; $P = 0.021$) than those with no evidence of HPV infections ($n = 56$). In contrast, HPV infections were not associated with OS in patients with risky oral habits

(HPV-positivity [$n = 182$] vs HPV-negativity [$n = 752$]: 70% vs 73%; $P = 0.187$).

Univariate and Multivariate Analyses

In univariate analysis, we identified the following clinicopathological factors as significantly associated with lower 5-year OS: histological differentiation, pathological tumor status, pathological lymph node status, pathological stage, histological tumor depth, ECS, and level IV/V metastases (2004 cohort; Table 3). No significant associations were found between OS and any HPV infection, HPV16 and other oncogenic HPV infections, HPV16 infection, and HPV18 infection. However, additional univariate analysis with stratified data revealed that, among patients with advanced OCC (pathological stage III/IV), the presence of HPV16 infection ($n = 23$) was associated with a significantly worse 5-year OS ($n = 234$; 34% vs 56% in those without HPV16 infection; $P = 0.038$). These results suggest that HPV16 infection was not a significant prognostic factor in univariate analysis because of confounding effects; therefore, HPV16 infection was entered into the multivariate regression model.

Correlation analyses between clinicopathological variables showed that all of the pathological factors were significantly correlated with each other, whereas HPV16 infection was not associated with any pathological factor. The results of multivariate analyses identified 4 adverse independent prognostic factors for OS (T2, T3, and T4 status; pathological N1 and N2 status; ECS; and HPV16 infection; Table 3).

In the 2008 cohort (Table 3), the same clinicopathological factors (histological differentiation, pathological tumor status, pathological lymph node status, pathological stage, histological tumor depth, ECS, and level IV/V metastases) were identified as being significantly associated with lower 5-year OS in univariate analyses. When these variables and HPV infections were entered as covariates into a multivariate regression model, pathological lymph node status (N1 and N2) and histological tumor depth (9–15 mm and 16–80 mm) were found to independently predict 5-year OS.

Prognostic Scoring System

For translational purposes, we further dichotomized continuous variables according to their optimal cut-off values (age >50 y; tumor depth >8 mm) in the 2004 cohort (model 1; Table 4). Multivariate analysis demonstrated that 4 factors were independent adverse prognostic predictors of 5-year OS: pathological T3/T4 status (HR 2.4 [95% CI 1.7–3.4]); pathological N1/N2 status (HR 2.9 [95% CI 1.8–4.5]); ECS (HR 1.9 [95% CI 1.2–3.0]); and HPV16 infection (HR 1.7 [95% CI 1.0–2.8]). In the 2008 cohort (model 2), 3 factors were independent adverse prognostic predictors for 5-year OS: pathological N1/N2 status (HR 2.4 [95% CI 1.5–3.9]); pathological stage III/IV (HR 2.0 [95% CI 1.0–4.1]); and histological tumor depth >8 mm (HR 2.1 [95% CI 1.3–3.5]). The weighting was based on a simple algorithm assigning the integer value of the corresponding HR to each factor (ie, 2 point for HR 1.5–2.4; 3 points for HR 2.5–3.4).

Specifically, a score of 2 was assigned in presence of pathological T3/T4 status, ECS, or HPV16 infection, and a score of 3 was given for pathological N1/N2 status (model 1). The resulting prognostic score (ranging from 0 to 9) was then applied to stratify the study cohort into 4 distinct prognostic groups, as follows: low (score 0 [40%], intermediate (score 1–3 [31%]), high (score 4–6 [14%]), and very high (score 7–9

TABLE 1. General Characteristics of Patients With Oral Cavity Cancer According to the Presence of Human Papillomavirus Infections

	Entire Cohort	HPV Status		
Variables	(n = 1002)	Negative (n = 808)	Positive (n = 194)	*P* value
Sex				
Female	64 (6%)	52 (6%)	12 (6%)	0.898
Male	938 (94%)	756 (94%)	182 (94%)	
Age groups (y; range 25−89 y)				
≤40	248 (25%)	200 (25%)	48 (25%)	0.400
41−50	223 (22%)	178 (22%)	45 (23%)	
51−60	318 (32%)	265 (33%)	53 (27%)	
>60	213 (21%)	165 (20%)	48 (25%)	
Alcohol drinking				
No	246 (25%)	203 (25%)	43 (22%)	0.420
Yes	756 (75%)	605 (75%)	151 (78%)	
Betel quid chewing				
No	167 (17%)	136 (17%)	31 (16%)	0.656
Yes	835 (83%)	672 (83%)	163 (84%)	
Cigarette smoking				
No	158 (16%)	128 (16%)	30 (16%)	0.908
Yes	844 (84%)	680 (84%)	164 (85%)	
Tumor subsite				
Tongue	322 (32%)	265 (33%)	57 (29%)	0.852
Floor of mouth	31 (3%)	24 (3%)	7 (4%)	
Lip	35 (4%)	26 (3%)	9 (5%)	
Cheek mucosa	398 (40%)	323 (40%)	75 (39%)	
Gum	149 (15%)	118 (15%)	31 (16%)	
Hard palate	12 (1%)	10 (1%)	2 (1%)	
Retromolar area	55 (6%)	42 (5%)	13 (7%)	
Histological differentiation				
Verrucous carcinoma	60 (6%)	50 (6%)	10 (5%)	0.240
Well differentiated	322 (32%)	268 (33%)	54 (28%)	
Moderately differentiated	527 (53%)	419 (52%)	108 (66%)	
Poorly differentiated	93 (9%)	71 (9%)	22 (11%)	
Pathological tumor status				
T1	183 (18%)	143 (18%)	40 (21%)	0.614
T2	408 (41%)	334 (41%)	74 (38%)	
T3	139 (14%)	115 (14%)	24 (12%)	
T4	272 (27%)	216 (27%)	56 (29%)	
Pathological lymph node status				
N0*	640 (64%)	521 (65%)	119 (61%)	0.627
N1	131 (13%)	102 (13%)	29 (15%)	
N2	231 (23%)	185 (23%)	46 (24%)	
Pathological stage				
I	156 (16%)	122 (15%)	34 (18%)	0.437
II	273 (27%)	229 (28%)	44 (23%)	
III	179 (18%)	143 (18%)	36 (20%)	
IV	394 (39%)	314 (39%)	80 (41%)	
Histological tumor depth (mm; range 0−80 mm)				
0−4	259 (26%)	218 (27%)	41 (21%)	0.355
5−8	245 (25%)	195 (24%)	50 (26%)	
9−15	271 (27%)	212 (26%)	59 (30%)	
16−80	227 (23%)	183 (23%)	44 (23%)	
Extracapsular spread				
No	794 (79%)	645 (80%)	149 (77%)	0.355
Yes	208 (21%)	163 (20%)	45 (23%)	
Level IV/V metastases				
No	980 (98%)	791 (98%)	189 (97%)	0.686
Yes	22 (2%)	17 (2%)	5 (3%)	

HPV = human papillomavirus.
Data are expressed as counts (%).
* Patients who did not undergo neck dissection because of clinical N0 disease (n = 114) were classified as having pathological N0 disease.

TABLE 2. Prevalence of Human Papillomavirus Infections in Oral Cavity Cancer

Subgroup/Genotype	Prevalence* (n = 1002)	Genotype	Prevalence* (n = 1002)
Any HPV type	194 (19%)	32	0
Single infection	169 (17%)	37	0
Multiple infection	25 (2%)	42	4 (<1%)
Oncogenic types	158 (16%)	43	2 (<1%)
16	80 (8%)	44	6 (1%)
18	51 (5%)	53	9 (1%)
31	2 (<1%)	54	0
33	2 (<1%)	55	3 (<1%)
35	2 (<1%)	61	1 (<1%)
39	0	62	8 (1%)
45	3 (<1%)	66	4 (<1%)
51	8 (1%)	67	0
52	17 (2%)	69	1 (< 1%)
56	2 (<1%)	70	4 (< 1%)
58	14 (1%)	71	3 (< 1%)
59	1 (<1%)	72	4 (< 1%)
68	1 (<1%)	74	0
HPV16/18	124 (12%)	81	6 (1%)
Other oncogenic types	34 (3%)	82	1 (< 1%)
Nononcogenic types	36 (4%)	83	0
6	0	84	3 (< 1%)
11	0	L1AE5	1 (< 1%)
26	0		

HPV = human papillomavirus.
* Data are expressed as counts (%).

[16%]; Table 5) risk. Patients with a low risk had an excellent 5-year OS (89%). However, the 5-year OS rates of patients with intermediate, high, and very high risk were 76%, 50%, and 25%, respectively (*P* < 0.005 for all comparisons). The c-index was 0.76, suggesting a satisfactory prediction performance.

A score of 2 was assigned in presence of each of the following risk factors: pathological N1/N2 status; pathological stage III/IV; and histological tumor depth >8 mm. The resulting prognostic score (ranging between 0 and 6) was applied to stratify the 2008 study cohort into 4 distinct prognostic groups, as follows: low risk (score 0 [30%]), intermediate risk (score 1−2 [18%]), high risk (score 3−4 [24%]), and very high risk (score 5−6 [28%]) (Table 5). Patients with a low risk had an excellent 5-year OS (95%). The 5-year OS rates of patients with intermediate, high, and very high risk were 83%, 74%, and 54%, respectively (*P* < 0.005 for all comparisons, the only exception being intermediate vs high [*P* = 0.152]). The c-index was 0.73 (indicating a satisfactory prediction performance of model 2).
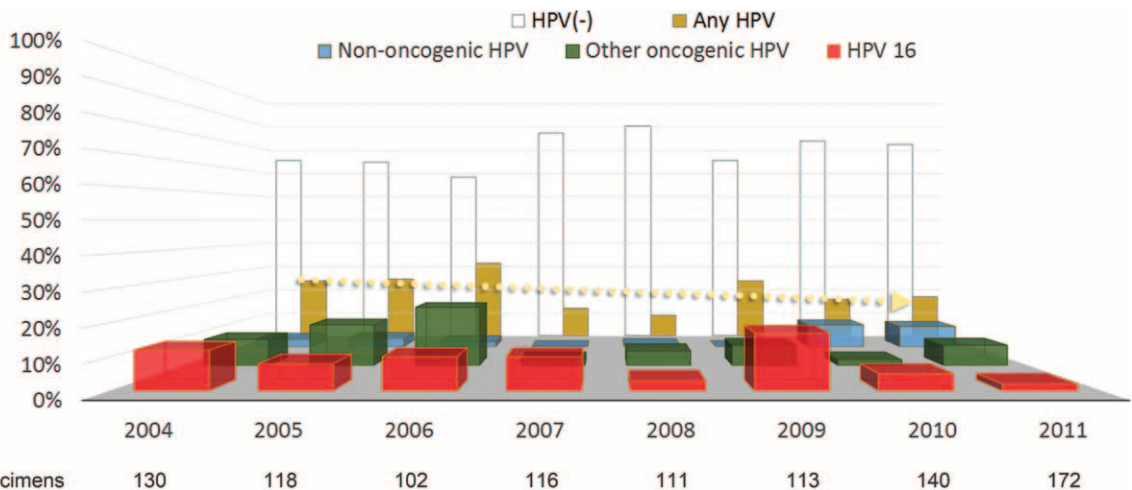

FIGURE 1. Prevalence of human papillomavirus (HPV) infections in patients with oral cavity cancer between 2004 and 2011. The dotted line indicates the prevalence trend for any HPV infection across the examined calendar-years. The number of specimens assessed for each assay is shown below the X-axis.

TABLE 3. Variables Associated With Risk of Death in the 2004 Cohort (n = 466) and 2008 Cohort (n = 536)

Variables	2004 Cohort				2008 Cohort			
			Overall Mortality (n = 143)				Overall Mortality (n = 110)	
	Number (%)	Number of Deaths	Univariate Analysis[*]	Multivariate Analysis[*]	Number (%)	Number of Deaths	Univariate Analysis[*]	Multivariate Analysis[*]
Sex								
Female	32 (7)	7	Reference	NA	32 (6)	5	Reference	NA
Male	434 (93)	136	1.6 (0.7−3.4)	NA	504 (94)	105	1.2 (0.5−3.0)	NA
Age groups (y)								
≤40	126 (27)	38	Reference	NA	122 (23)	25	Reference	NA
41−50	112 (24)	38	1.1 (0.7−1.7)	NA	111 (21)	23	1.0 (0.6−1.8)	NA
51−60	126 (27)	32	0.8 (0.5−1.3)	NA	192 (36)	33	0.8 (0.5−1.4)	NA
>60	102 (22)	35	1.1 (0.7−1.8)	NA	111 (21)	29	1.5 (0.9−2.5)	NA
Alcohol drinking								
No	136 (29)	37	Reference	NA	111 (21)	23	Reference	NA
Yes	330 (71)	106	1.2 (0.8−1.8)	NA	425 (79)	87	1.0 (0.6−1.5)	NA
Betel quid chewing								
No	80 (17)	23	Reference	NA	87 (16)	19	Reference	NA
Yes	386 (83)	120	1.1 (0.7−1.8)	NA	449 (84)	91	0.9 (0.6−1.5)	NA
Cigarette smoking								
No	71 (15)	17	Reference	NA	87 (16)	14	Reference	NA
Yes	395 (85)	126	1.5 (0.9−2.4)	NA	449 (84)	96	1.3 (0.7−2.3)	NA
Tumor subsite								
Tongue	146 (31)	50	Reference	NA	176 (33)	33	Reference	NA
Floor of mouth	17 (4)	7	1.1 (0.5−2.5)	NA	14 (3)	3	1.3 (0.4−4.1)	NA
Lip	14 (3)	3	0.5 (0.2−1.7)	NA	21 (4)	2	0.5 (0.1−2.0)	NA
Cheek mucosa	185 (40)	45	0.7 (0.4−1.0)	NA	213 (40)	51	1.4 (0.9−2.1)	NA
Gum	69 (15)	26	1.1 (0.7−1.8)	NA	80 (15)	18	1.3 (0.7−2.2)	NA
Hard palate	6 (1)	3	1.8 (0.6−5.9)	NA	6 (1)	0	0 (0−1.5 × e^{177})	NA
Retromolar area	29 (6)	9	0.9 (0.4−1.7)	NA	26 (5)	3	6.4 (0.2−2.1)	NA
Histological differentiation								
Well differentiated	135 (29)	27	Reference	NS	187 (35)	31	Reference	NS
Moderately differentiated	272 (58)	99	2.1 (1.4−3.2)	NS	255 (48)	57	1.4 (0.9−2.2)	NS
Poorly differentiated	34 (7)	14	2.6 (1.3−4.9)	NS	59 (11)	21	2.5 (1.4−4.3)	NS
Verrucous carcinoma	25 (5)	3	0.6 (0.2−1.9)	NS	35 (7)	1	0.1 (0.0−1.1)	NS
Pathological tumor status								
T1	90 (19)	7	Reference	Reference	93 (17)	8	Reference	NS
T2	194 (42)	47	3.4 (1.6−7.6)	2.5 (1.1−5.5)	214(40)	37	2.2 (1.0−4.6)	NS
T3	68 (15)	25	5.7 (2.5−13.2)	4.0 (1.7−9.4)	71 (13)	11	2.0 (0.8−4.9)	NS
T4	114 (25)	64	10.6 (4.9−23.2)	5.8 (2.6−12.9)	158 (30)	54	4.9 (2.3−10.3)	NS
Pathological lymph node status								
N0	294 (63)	47	Reference	Reference	346 (65)	37	Reference	Reference
N1	56 (12)	27	3.8 (2.4−6.1)	2.5 (1.5−4.2)	75 (14)	21	3.1 (1.8−5.2)	2.4 (1.4−4.1)
N2	116 (25)	69	5.8 (4.0−8.4)	2.8 (1.7−4.8)	115 (22)	52	5.4 (3.5−8.2)	3.7 (2.4−5.8)
Pathological stage								
I	79 (17)	5	Reference	NS	77 (14)	4	Reference	NS
II	130 (28)	20	2.5 (0.9−6.7)	NS	143 (27)	11	1.5 (0.5−4.8)	NS
III	79 (17)	24	5.4 (2.0−14.1)	NS	100 (19)	16	3.5 (1.2−10.5)	NS
IV	178 (38)	94	12.0 (4.9−29.4)	NS	216 (40)	79	8.9 (3.3−24.2)	NS
Histological tumor depth (mm)								
0−4	137 (29)	21	Reference	NS	122 (23)	8	Reference	Reference
5−8	133 (29)	34	1.8 (1.0−3.0)	NS	112 (21)	14	2.0 (0.8−4.9)	1.5 (0.6−3.5)
9−15	109 (23)	42	3.0 (1.8−5.1)	NS	162 (30)	38	4.3 (2.0−9.2)	2.4 (1.1−5.4)
16−80	87 (19)	46	4.8 (2.9−8.1)	NS	140 (26)	50	7.3 (3.5−15.5)	3.9 (1.8−8.7)
Extracapsular spread								
No	364 (78)	76	Reference	Reference	430 (80)	62	Reference	NS
Yes	102 (22)	67	5.0 (3.6−6.9)	1.8 (1.1−2.8)	106 (20)	48	3.9 (2.6−5.6)	NS
Level IV/V metastases								
No	454 (97)	134	Reference	NS	526 (98)	105	Reference	NS
Yes	12 (3)	9	5.2 (2.6−10.1)	NS	10 (2)	5	3.0 (1.2−7.2)	NS
Any HPV infection								
Negative	360 (77)	105	Reference	NA	448 (84)	92	Reference	NA
Positive	106 (23)	38	1.3 (0.9−1.9)	NA	88 (16)	18	1.0 (0.6−1.6)	NA
HPV status								
Negative	360 (77)	105	Reference	NA	448 (84)	92	Reference	NA
Nononcogenic types	10 (2)	0	0 (0−2.1 × e^{177})	NA	26 (5)	6	1.3 (0.6−2.9)	NA
Other oncogenic types	49 (11)	19	1.5 (0.9−2.4)	NA	29 (5)	5	0.8 (0.3−1.9)	NA
HPV16	47 (10)	19	1.5 (0.9−2.4)	NA	33 (6)	7	1.0 (0.5−2.2)	NA
HPV16 infection								
Negative	419 (90)	124	Reference	Reference	503 (94)	103	Reference	NS
Positive	47 (10)	19	1.4 (0.9−2.3)	1.6 (1.0−2.7)	33 (6)	7	1.0 (0.5−2.2)	NS
HPV18 infection								
Negative	433 (93)	128	Reference	NA	518 (97)	110	Reference	NA
Positive	33 (7)	15	1.6 (0.9−2.7)	NA	18 (3)	0	0.0 (0.0−3.4)	NA

HPV = human papillomavirus; NA = not available for multivariate analysis; NS = not significant based on multivariate analysis.
[*] Data are expressed as hazard ratios (95% confidence interval).

TABLE 4. Multivariate Analysis of Variables Predicting 5-Year Overall Survival

		Model 1 (2004 Cohort, n = 466)			Model 2 (2008 Cohort, n = 536)		
		Overall Mortality (n = 143), Multivariate Analysis			Overall Mortality (n = 110), Multivariate Analysis		
Variables	Number	Log Hazard Ratio*	P Value	Relative Risk	Number	Log Hazard Ratio*	P Value	Relative Risk
Male sex	434	NA			504	NA		
Age >50 y	531	NA			414	NA		
Alcohol drinking	380	NA			425	NA		
Betel quid chewing	336	NA			449	NA		
Cigarette smoking	395	NA			449	NA		
Moderate/poor differentiation	306	NS			314	NS		
Pathological T3/T4 status	182	0.9 ± 0.2	<0.001	2.4	229	NS		
Pathological N1/N2 status	172	1.0 ± 0.2	<0.001	2.9	190	0.9 ± 0.2	<0.001	2.4
Pathological stage III/IV	257	NS			316	0.7 ± 0.4	0.040	2.1
Histological tumor depth >8 mm	196	NS			414	0.7 ± 0.3	0.004	2.1
Extracapsular spread	102	0.7 ± 0.2	0.004	1.9	106	NS		
Level IV/V metastases	12	NS			10	NS		
Any HPV infection	106	NS			88	NS		
Nononcogenic HPV infection	10	NA			26	NA		
Oncogenic HPV infection	96	NS			62	NS		
HPV16 infection	47	0.5 ± 0.2	0.032	1.7	33	NS		
HPV18 infection	33	NA			18	NA		

CI = confidence interval; HPV = human papillomavirus; NA = not available for multivariate analysis; NS = not significant based on multivariate analysis.
*Data are expressed as log hazard ratio ± standard error and hazard ratios (95% CI).

The analysis of OS in the 2008 cohort also demonstrated the internal validity of the model 1 system for the main endpoint. The 5-year OS rates of the 4 risk groups were 92%, 77%, 57%, and 51%, respectively ($P < 0.001$; Table 5), with a c-statistic of 0.71. Subgroup analyses corroborated the discriminative strength of the prognostic scoring system for all comparisons, the only exception being the very-high–risk group compared with the high-risk group ($P = 0.216$). As the prevalence of HPV16 infection significantly decreased from 10% (2004 cohort) to 6% (2008 cohort), the proportion of HPV16 infections was similarly reduced from 19% to 10% in the very-high–risk group. The proportion of the very-high–risk group was also reduced from 16% to 13% in model 1, albeit not significantly so ($P = 0.121$ and 0.461, respectively). As long as risk scores were analogous, similar survival rates were observed. This phenomenon caused a decreased discriminative strength for the comparison between the very high and high-risk groups in the 2008 cohort. The internal validity of model 2 for the main outcome measure was investigated in the 2004 cohort. The 5-year OS rates of the 4 risk groups were 87%, 87%, 64%, and 36%, respectively ($P < 0.001$; c-statistic = 0.74; Table 5). Only the low and intermediate-risk groups did not differ from each other ($P = 0.920$). All other comparisons were statistically significant.

Comparison Between Prognostic Scoring System and Sixth Edition AJCC Stage System

The 5-year OS differed significantly according to the traditional AJCC pathological stage in the 2004 (Table 3) and 2008 (Table 5) cohorts, the only exception being a marginal difference between patients with a pathological stage of 2 (85%

and 91%, respectively) and those with a pathological stage of 1 (94% and 94%, respectively; $P = 0.053$ and 0.68, respectively). The c-statistics for pathological stage were 0.72 for the 2004 cohort and 0.70 for the 2008 cohort, respectively. The Kaplan–Meier survival curves for OS according to the pathological stage and the model 1 and model 2 systems in the entire cohort are shown in Figure 2. In the entire study cohort, the c-statistics for pathological stage, model 1, and model 2 were 0.71, 0.73, and 0.72, respectively.

DISCUSSION

The results of the present study indicate that HPV infections are common among Taiwanese OCC patients, this finding being in line with a recent meta-analysis (pooled HPV-DNA prevalence 24%).[22] However, the prevalence of all HPV infections, oncogenic HPV infections, and both HPV16 and HPV18 infections decreased significantly between 2004 and 2011. In contrast, nononcogenic HPV infections showed an increasing trend in the same calendar period. Importantly, we demonstrate that HPV16 infection is an independent adverse prognostic factor for OS in OCC patients. To our knowledge, this study is the first to internally validate a prognostic index that included HPV16 infection for predicting 5-year OS in OCC patients. The index had a high discriminatory power and was a significant predictor of prognosis at the individual patient level. However, further external validation is required before it could be implemented in clinical practice.

A better understanding of the pathogenic role of HPV infections in OCC patients is needed for assessing the potential utility of HPV vaccination campaigns. The reported prevalence of oncogenic HPV infections in apparently healthy US adults

TABLE 5. Prognostic Scoring System in Relation to 5-Year Overall Survival

Risk Group	Score	Model 1				Score	Model 2			
		Patients (n [%])	5-Year OS	95% CI*	HR (95% CI)		Patients (n [%])	5-Year OS	95% CI*	HR (95% CI)
Prognostic scoring system	2004 cohort					2008 cohort				
Low	0	185 (40%)	89%	84%–94%†	Reference	0	160 (30)	95%	92%–98%‖	Reference
Intermediate	1–3	143 (31%)	76%	69%–83%†	2.3 (1.3–3.9)	1–2	95 (18)	83%	75%–91%‖	3.5 (1.4–8.8)
High	4–6	66 (14%)	50%	38%–62%†	5.9 (3.4–10.1)	3–4	130 (24)	74%	64%–84%‖	5.6 (2.4–12.9)
Very high	7–9	72 (16%)	25%	15%–35%†	13.1 (7.9–21.7)	5–6	151 (28)	54%	45%–63%‖	13.2 (6.0–28.8)
Internal validation	2008 cohort					2004 cohort				
Low	0	204 (38%)	92%	88%–96%‡	Reference	0	177 (38)	87%	82%–92%¶	Reference
Intermediate	1–3	186 (35%)	77%	69%–85%‡	2.8 (1.5–5.1)	1–2	63 (14)	87%	79%–95%¶	1.0 (0.4–2.2)
High	4–6	77 (14%)	57%	43%–71%‡	6.4 (3.4–11.9)	3–4	116 (25)	64%	56%–72%¶	3.2 (1.9–5.3)
Very high	7–9	69 (13%)	51%	39%–63%‡	8.6 (4.7–15.9)	5–6	110 (24)	36%	27%–45%¶	8.2 (5.1–13.1)
Traditional AJCC stage	2008 cohort					2004 cohort				
Stage I		77 (14%)	94%	88%–100%§	Reference		79 (17)	94%	89%–99%#	Reference
Stage II		143 (27%)	91%	86%–96%§	1.5 (0.5–4.8)		130 (28)	85%	79%–91%#	2.5 (0.9–6.7)
Stage III		100 (19%)	76%	63%–90%§	3.5 (1.2–10.5)		79 (17)	70%	60%–80%#	5.4 (2.0–14.1)
Stage IV		216 (40%)	60%	53%–67%§	8.9 (3.3–24.4)		178 (38)	47%	40%–54%#	12.0 (4.9–29.4)

AJCC = American Joint Committee on Cancer; CI = confidence interval; HR = hazard ratio; OS = overall survival; SE = standard error.

* 95% CI of the estimated survival rate equals the estimated survival rate $\pm 1.96 \times$ SE.

† Pairwise comparisons of OS between patients with different risks were found to be significantly different ($P < 0.05$).

‡ Pairwise comparisons of OS between patients with different risks were found to be significantly different ($P < 0.05$).

§ Pairwise comparisons of OS between patients with different risks were found to be significantly different ($P < 0.05$).

‖ Pairwise comparisons of OS between patients with different risks were found to be significantly different ($P < 0.05$), whereas high versus very high did not differ significantly ($P = 0.216$).

¶ Pairwise comparisons of OS between patients with different risks were found to be significantly different ($P < 0.05$), whereas stage 1 versus stage 2 did not differ significantly ($P = 0.468$).

Pairwise comparisons of OS between patients with different risks were found to be significantly different ($P < 0.05$), whereas intermediate versus high did not differ significantly ($P = 0.152$).

Pairwise comparisons of OS between patients with different risks were found to be significantly different ($P < 0.05$), whereas low versus intermediate did not differ significantly ($P = 0.920$).

Pairwise comparisons of OS between patients with different risks were found to be significantly different ($P < 0.05$), whereas stage 1 versus stage 2 did not differ significantly ($P = 0.053$).
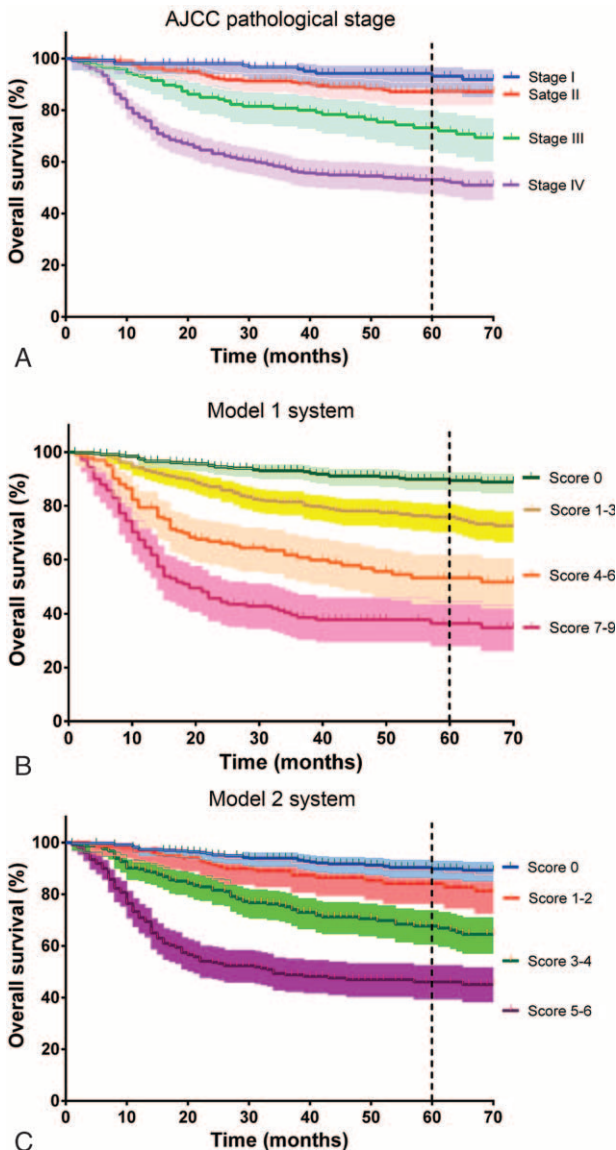
FIGURE 2. Kaplan–Meier estimates of the 5-year overall survival in patients with oral cavity cancer (n = 1002). A, Risk stratification according to the 2002 American Joint Committee on Cancer (AJCC) pathological stage (log-rank test, $P < 0.001$). B, Risk stratification according to model 1 ($P < 0.001$). C, Risk stratification according to model 2 ($P < 0.001$). Shallow areas indicate the 95% confidence interval (CI).

aged between 25 and 69 years is 2% to 7%.[23] Most of our knowledge about HPV epidemiology relates to the oropharynx, where HPV infections are becoming more prevalent, tend to affect younger people, and are associated with a better prognosis than HPV-negative tumors.[24–26] In this large study conducted in OCC patients, we have shown that HPV infections tended to occur less frequently during the past decade, being fairly stable across different age groups. However, we cannot exclude the occurrence of year-to-year variations, ultimately requiring data at 10 years to clearly establish a temporal trend. Notably, HPV-positive tumors were associated with a worse OS, especially in patients without risky oral habits. Among the

HPV types (n = 158), HPV16 was the most commonly observed among our OCC patients (51% [n = 80]), although at a significantly lower frequency than that previously reported for HPV-positive OPC (>90%).[27] HPV18 (26% [n = 51]) and HPV58 (7% [n = 14]) were other frequently encountered oncogenic types in this study.

Human papillomavirus molecular oncogenesis is significantly different from that of tobacco and alcohol-related head and neck malignancies in terms of genetic mutations or deletions.[28] The superior response to treatment in HPV-positive OPC patients may result from a higher likelihood of restoring a normal cell growth control.[25] The observation that HPV infections are related to a worse prognosis in OCC patients without risky oral habits suggests that detection of intratumor HPV DNA may not characterize the tumor as being caused by the virus. The use of p16-based assays can detect oncogenic HPV activity,[29] but immunohistochemical analysis of p16 expression lacks prognostic utility in OCC patients, unless its intracellular localization is considered.[28] A more recent study suggested that a combined p16/HPV testing is necessary to identify HPV-associated nonoropharyngeal head and neck cancers characterized by favorable outcomes.[29] However, the detection of HPV-DNA using PCR and p16 expression in cancer biopsies lacks specificity, and HPV E6/E7 antibody detection lacks sensitivity.[30,31] Therefore, such tests remain suboptimal for the detection of HPV infections.[2] Moreover, the genetic diversity of OCC patients (regardless of HPV infections) may play a key role in determining prognosis and treatment outcomes. In our previous studies, several molecular markers, including $G\alpha_{12}$,[32] crucial upstream drivers,[33] miRNA-491-5p and GIT1,[34] BST2,[35] oncomiR-196,[36] and somatic copy numbers,[37] have been linked to clinical outcomes in small case series. Studies aimed at investigating the prognostic impact of genetic variants in larger clinical cohorts are currently ongoing.

These caveats notwithstanding, our results suggest that HPV16 infections are independently associated with a lower OS in OCC patients. In previous smaller studies, we have shown that the detection of HPV16 DNA in patients with advanced OCC[12] and high HPV16/18 E7 viral load in OCC patients[16] are related to distant metastasis and OS. We further developed a prognostic scoring system that incorporates both common prognostic factors and the presence of HPV16 infections for predicting OS in a more accurate manner than traditional pathological stage. The results indicated that the projected 5-year OS rates were 89% for low-risk, 76% for intermediate-risk, 50% for high-risk, and 25% for very-high-risk patients, respectively. One potential strategy to improve outcomes in patients at high risk of death after combination therapy (ie, those with a prognostic score ≥ 7) may rely on a more aggressive initial treatment, based on intensive chemoradiation regimens. Such a stratification system might be useful as part of the prognostic evaluation in OCC patients and as an enrichment strategy for clinical trials.

Recently, the International Consortium for Outcome Research in Head and Neck Cancer has attempted to review the prognostic performance of the AJCC classification of OCC. The results indicated that the inclusion of histological tumor depth (depth of invasion) can improve T-staging system.[38] Moreover, the number of metastatic lymph nodes in patients with both N2b and N2c may improve the prognostic performance of the N-staging system.[39] Finally, T3N1 and stage IVa disease in OCC have been shown to be similar.[40] The results of the current study indicate that ECS and HPV16 can help refining the prognostication of OCC patients in model 1

(distinction between low vs intermediate risk, and high vs very high risk). Moreover, histological tumor depth can help identifying high-risk patients in model 2. On the basis of the similar c-statistics, we suggest that these categories could be grouped together in future revisions of the AJCC staging system with the overall goal of enhancing prognostic accuracy. In contrast, the validation 2008 cohort had a decreased prevalence of HPV16 infection and a favorable OS in the very-high–risk group, suggesting the clinical usefulness of preventing HPV16 infections. Since 2 licensed prophylactic vaccines against HPV16 and HPV18 has been marketed for almost a decade,[41,42] it is expected that both the prevalence of HPV infections and their impact on OS of OCC patients will be declining in the upcoming decades.

In summary, our findings suggest that HPV16 infections may increase the risk of death by any cause in high-risk OCC patients. If independently confirmed, the prognostic score may be useful for allocating OCC patients to risk-adapted therapies in countries with a high prevalence of HPV infections. However, patients with HPV-positive OCC should be currently treated using standard-of-care approaches unless otherwise demonstrated in future studies. Because of potential biases (eg, ethnical differences, variations in risky oral habits, retrospective nature of the study, different laboratory assays for detecting HPV infections), our data need to be validated in ethnically diverse populations. This cohort study provides a strong rationale for implementing such collaborative efforts. Larger studies focusing on a longer time period are warranted to confirm the declining trend of HPV infections.

ACKNOWLEDGMENTS

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