Research Article

Cancer Epidemiology, Biomarkers & Prevention

Prevalence, Clinicopathological Characteristics, and Outcome of Human Papillomavirus–Associated Oropharyngeal Cancer in Southern Chinese Patients

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Abstract

Background: Although the global incidence of human papillomavirus (HPV)–associated oropharyngeal squamous cell carcinoma (OPSCC) is increasing, there is little information on southern Chinese population available.

Methods: We analyzed 207 patients which constituted 63.5% of all newly diagnosed OPSCC in Hong Kong during a 5-year period from 2005 to 2009.

Results: We used E6/7 mRNA as a marker of oncogenic involvement and found 20.8% (43/207) of OPSCC and 29.0% (36/124) of tonsillar SCC was associated with HPV. HPV-16 was identified in all cases except one (HPV-18). Patients with HPV-associated OPSCCs were significantly younger than HPV-negative patients (mean age: 59.8 vs. 63.9 years, $P = 0.05$). Multivariate analysis showed that HPV-associated OPSCC was more likely to occur in nonsmokers (39.5% vs. 15.1%, OR: 2.89, $P = 0.05$), nondrinkers (52.5% vs. 25.6%, OR: 2.72, $P = 0.04$), originate from the palatine tonsils (83.7% vs. 53.7%, OR: 3.88, $P = 0.01$), present with an early primary tumor (T1/2; 79.1% vs. 47.6%, OR: 3.81, $P = 0.004$), and exhibit basaloid differentiation (33.3% vs. 7.3%, OR: 19.74, $P = 0.006$). HPV positivity was an independent predictor for better prognosis for both 5-year overall and 5-year disease-specific survivals (DSS; 63.0% vs. 29.7%, HR: 0.33, $P < 0.001$, and 87.8% vs. 42.6%, HR: 0.16, $P < 0.001$, respectively).

Conclusion: The estimated age-standardized incidence of OPSCC in Hong Kong during the period 2005–2009 was 0.12/100,000/year.

Impact: This study has provided the most comprehensive clinical and pathologic information to date about this newly recognized disease in southern Chinese. In view of the global trend, we should anticipate and prepare for an increase in HPV-related OPSCC in southern China. *Cancer Epidemiol Biomarkers Prev; 25(1); 165–73. ©2015 AACR.*

Introduction

Human papillomavirus (HPV)–associated oropharyngeal squamous cell carcinoma (OPSCC) is widely accepted as an etiologically distinct head and neck cancer (1, 2). It was first postulated by Stina Syrjanen in 1983 who observed morphologic and IHC features of viral infection in oral cancer (3). Eventually the causative role of HPV in OPSCC was declared by the World Health Organization in 2007 (4). Despite the decreasing trend in the prevalence of smoking and the subsequent decreasing incidence of all head and neck cancers over the past three decades (5), the incidence of oropharyngeal cancer in European and American populations has doubled (6, 7). Similar trends of its incidence have also been observed in many countries worldwide, especially in the economically developed countries (8).

The proportion of OPSCC associated with HPV is highly variable across different countries (7, 9). In contrast with the West, there has been relatively little interest from Asian nations on this newly recognized disease. In Asia, the reported prevalence of HPV in oropharyngeal cancer ranges from 0% to 34.4% (9–12). Similar to those reported from Europe and

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Note: Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (http://cebp.aacrjournals.org/).

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doi: 10.1158/1055-9965.EPI-15-0869

North America, a recent multicenter study from Korea showed an increase in the proportion of tonsillar SCC due to HPV from 5.9% in 1991 to 31.6% in 2009 (13, 14). Information on HPV-associated oropharyngeal cancer in Hong Kong, with a mixed European and Chinese culture, is very limited. In order to better determine the importance and significance of this disease in Hong Kong, we conducted a population-based multicenter study to determine the prevalence, clinicopathological characteristics, and outcomes of HPV-associated OPSCC in a southern Chinese population living in a cosmopolitan city in East Asia.

Materials and Methods

Case identification and verification

All new cases of OPSCC diagnosed between 2005 and 2009 in public hospitals in Hong Kong were identified from hospital electronic records. To estimate the coverage of our patient pool, the patient list was verified with the database of the Hong Kong Cancer Registry. On the basis of the International Classification of Diseases version 9 (ICD-9), tumors in all oropharyngeal subsites were included. This encompassed malignant neoplasms of the oropharynx (ICD-9-146), base of tongue (ICD-9-141.1), lingual tonsils (ICD-9-141.6), and soft palate (ICD-9-145.3). The anatomical codes were verified with all available clinical and imaging records. The definition of squamous cell carcinoma was based on the International Classification of Diseases for Oncology (ICD-O-2), and included M8050 to M8082. Hematoxylin and eosin (H&E)-stained slides were prepared from the formalin-fixed paraffin-embedded (FFPE) tumor tissues and were verified by two consultant pathologists according to the above definition to determine the eligibility of the potential cases. Patients with completed clinical records and available tumor tissues for further verification of the diagnosis were included.

Epidemiologic data and tumor staging

Demographic data, marital status, smoking and drinking habits, history of head and neck malignancies and treatment modalities, as well as the occurrence of second primary tumors were collected from both written and electronic clinical records. All operative findings were reviewed by the authors and all available radiologic investigations were reviewed by a radiologist to verify the stage of the disease, which was classified according to the American Joint Cancer Committee (AJCC), the seventh edition in 2010 (15).

Histologic features

The most representative FFPE tumor tissue cell blocks were selected for further analysis. One H&E slide was prepared for the prospective verification of the histologic diagnosis by two pathologists separately. Subjects with the diagnosis of non-SCC or where there was an absence of tumor cells were excluded. The final histologic diagnoses made by the two pathologists were compared. The discordant results were further analyzed. Hence, the risk of misclassified subjects in our study population should be minimal.

The grade of differentiation, degree of keratinization, severity of necrosis, degree of nuclear pleomorphism, and features of basaloid differentiation, including high nuclear to cytoplasmic (N/C) ratio, solid growth pattern, and peripheral palisading as described by Cooper and colleagues (2013) were determined (16).

HPV and p16 status

Previously described procedures were used for DNA extraction and DNA quality assessment (17, 18). HPV DNA was detected by PCR using the consensus primers GP5+/GP6+_52HK which has a reserve primer modified based on the HPV52 variants circulating in Hong Kong (19). HPV type was identified by sequencing of the PCR amplicons.

To assist with analysis on the oncogenic role of any high-risk HPV found, the expression of mRNA encoded viral oncoproteins E6/E7 was examined. The most predominant spliced E6/E7 mRNA, E6*I mRNA, was measured in this study. RNA was extracted from FFPE tissue using the QIAGEN RNeasy FFPE kit (Qiagen). The quality of extracted RNA preparation was assessed by real-time PCR with primers and probes targeting specifically the splicing region of mRNA encoded by housekeeping gene RPS18: forward primer (RPS18-F125) – CCT TTG CCA TCA CTG CCA TT, reverse primer (RPS18-R254) – ACT GGC GTG GAT TCT GCA TAA, probe (RPS18-224P) – 5′FAM CTG AGG ATG AGG TGG AAC 3′MGBNFQ. The qRT-PCR reaction contained 2 μL of purified RNA, 0.25 μL of SuperScript III/Platinum T*aq* Mix (Life Technologies), and 12.5 μL of 2× Reaction Mix in a final reaction volume of 25 μL. The primer and probe concentrations were 0.3 μM and 0.2 μM, respectively. The cycling conditions were 50°C for 30 minutes, 95°C for 2 minutes, followed by 40 cycles of 95°C for 15 seconds and 60°C for 30 seconds using the SuperScript III Platinum One-Step qRT-PCR Kit w/ROX (Life Technologies).

Primers and probes specifically targeted to cross the splicing sites of E6*I mRNA were designed, HPV16 E6*I: forward primer (16mE6_1_F) - GAA TGT GTG TAC TGC AAG CAA CAG, reverse primer (16mE6_1_R) – TCA GGA CAC AGT GGC TTT TGA C, probe (E6*I-P) – 5′FAM CGA CGT GAG GTG TAT TAA 3′MGBNFQ. HPV18 E6*I forward primer (18E6-27F): GCGACCCTACAAGCTACCTGAT, HPV18 E6*I reverse primer (18E6-177R): TGTCTAAGTTTTTCTGCTGGATTCA, HPV18 E6*I probe (18E6-119P): 5′FAM AACTTACAGAGGTGCCTGC 3′MGBNFQ. The qRT-PCR reaction is described above. The primer and probe concentration was 0.4 and 0.2 μM, respectively, for both HPV-16 and HPV-18. The cycling conditions were 50°C for 15 minutes, 95°C for 2 minutes, followed by 40 cycles of 95°C for 15 seconds, and 55°C (for HPV-16, or 58°C for HPV18) for 30 seconds using the SuperScript III Platinum One-Step qRT-PCR Kit w/ROX (Life Technologies).

The CINtec p16 monoclonal antibody Clone E6H4 (Ventana, U.S.A) was used for p16 staining by IHC. Slides were assessed by two pathologists who were blinded from the clinical information and HPV testing results. The scoring system was based on the stained pattern and the percentage of stained tumor cells in the slides. The score was categorized as 0, 1+, 2+, and 3+ (negative, sporadic, focally and diffusely positive respectively). Subsequently, the p16 immunostaining result was dichotomized as "p16-positive" when it scored 3+. Otherwise, it was recorded as "p16-negative".

Statistical analysis

The correlations between HPV-associated cancer and various characteristics were assessed by χ^2 test or Fisher's exact test as

appropriate. The ORs were determined by the univariate analysis using the binary logistic regression model. Multivariate analysis, using logistic regression to adjust the significant correlators identified in the univariate analysis, was used to determine the independent correlators. All survival analyses were assessed with the Kaplan–Meier analysis with the log-rank test. The independent prognostic factors were determined by the Cox regression model. In the overall survival (OS) analysis, patients who were still alive at the last follow-up or dropped out of the subsequent follow-up were censored. In the DSS analysis, additional patients whose deaths were not related to oropharyngeal cancer were censored. All statistical tests were two tailed and P values of ≤ 0.05 were considered significant. All data were analyzed using SPSS Version 20 (SPSS Inc.).

Table 1. Clinical characteristics of 207 patients with OPSCC in Hong Kong

	HPV-associated OPSCC ($N = 43$)/(%)	Non–HPV-associated OPSCC ($N = 164$)/(%)	Univariate analysis			Multivariate analysis[a]		
			OR	95% CI	*P*	OR	95% CI	*P*
Age								
Mean (SD)	59.8 (12.4)	63.9 (11.9)	−4.0[b]	−8.08–0.02	0.05			
Range	41–87	33–90						
Age group								
≥50 years	33 (76.7)	147 (89.6)	Ref.			Ref.		
<50 years	10 (23.3)	17 (10.4)	2.62	1.10–6.24	0.03	1.86	0.63–5.43	0.26
Gender								
Male	34 (79.1)	144 (87.8)	Ref.					
Female	9 (20.9)	20 (12.2)	1.91	0.80–4.55	0.15			
Smoking	*N = 43*	*N = 159*						
Ever smoker	26 (60.5)	135 (84.9)	Ref.			Ref.		
Non smoker	17 (39.5)	24 (15.1)	3.68	1.74–7.79	0.001	2.89	0.99–8.41	0.05
Drinking	*N = 40*	*N = 156*						
Ever drinker	19 (47.5)	116 (74.4)	Ref.			Ref.		
Non drinker	21 (52.5)	40 (25.6)	3.21	1.57–6.57	0.001	2.72	1.04–7.13	0.04
Marital status								
Single	4 (9.3)	17 (10.4)	Ref.					
Married/divorced	39 (90.7)	147 (89.6)	1.13	0.36–3.54	0.84			
History of head & neck cancer								
Yes	1 (2.3)	30 (18.3)	Ref.			Ref.		
No	42 (97.7)	134 (81.7)	9.40	1.25–71.05	0.03	4.41	0.26–75.94	0.31
History of head & neck irradiation								
Yes	1 (2.3)	28 (17.1)	Ref.			Ref.		
No	42 (97.7)	136 (82.9)	8.65	1.14–65.47	0.04	2.65	0.14–49.22	0.51
History of other malignancies								
No	40 (93.0)	153 (93.3)	Ref.					
Yes	3 (7.0)	11 (6.7)	1.04	0.28–3.91	0.95			
Primary site					0.04			
Palatine tonsils	36 (83.7)	88 (53.7)	Ref.					
Base of tongue	4 (9.3)	35 (21.3)	0.28	0.09–0.84	0.02			
Soft palate	3 (7.0)	29 (17.7)	0.25	0.07–0.88	0.03			
Other oropharyngeal walls	0 (0)	12 (7.3)	<0.001		1.00			
Origin: Palatine tonsil								
No	7 (16.3)	76 (46.3)	Ref.			Ref.		
Yes	36 (83.7)	88 (53.7)	4.44	1.87–10.56	0.001	3.88	1.36–11.05	0.01
Group staging					0.06			
Stage I	0 (0)	9 (5.5)						
Stage II	5 (11.6)	21 (12.8)						
Stage III	6 (14.0)	12 (7.3)						
Stage IVa	27 (62.8)	78 (47.6)						
Stage IVb	5 (11.6)	26 (15.9)						
Stage IVc	0 (0)	18 (11.0)						
Primary tumor								
Advanced primary tumor (T3/T4)	9 (20.9)	86 (52.4)	Ref.			Ref.		
Early primary tumor (T1/T2)	34 (79.1)	78 (47.6)	4.17	1.88–9.23	<0.001	3.81	1.55–9.39	0.004
Nodal status								
Nodal negative (N0)	5 (11.6)	55 (33.5)	Ref.			Ref.		
Nodal positive (N1–N3)	38 (88.4)	109 (66.5)	3.84	1.43–10.29	0.008	3.07	0.92–10.27	0.07
M-stage					0.02			
M0	43 (100)	146 (89.0)	Ref.					
M1	0 (0)	18 (11.0)	N.A.					
Synchronous tumors					0.08			
Yes	0 (0)	12 (7.3)	Ref.					
No	43 (100.0)	152 (92.7)	N.A.					

[a]M stage is not included in the multivariate analysis as none of the HPV-associated OPSCCs presented with distant metastasis, $R^2 = 36.1.\%$.
[b]Difference in mean age.

Table 2. Tumor staging and treatment modality according to HPV status

	HPV-associated OPSCC ($N = 43$)/(%)	Non–HPV-associated OPSCC ($N = 164$)/(%)	*P*
T-stage			<0.001
T1	15 (34.9)	18 (11.0)	
T2	19 (44.2)	60 (36.6)	
T3	0 (0)	23 (14.0)	
T4a	7 (16.3)	44 (26.8)	
T4b	2 (4.7)	19 (11.6)	
N-stage			0.03
N0	5 (11.6)	55 (33.5)	
N1	6 (14.0)	10 (6.1)	
N2a	4 (9.3)	8 (4.9)	
N2b	21 (48.8)	57 (34.8)	
N2c	5 (11.6)	19 (11.6)	
N3	2 (4.7)	15 (9.1)	
M-stage			0.03
M0	43 (100)	146 (89.0)	
M1	0 (0)	18 (11.0)	
Stage			0.25
I	9 (5.5)	0 (0)	
II	21 (12.9)	5 (11.6)	
III	12 (7.4)	6 (14.0)	
IV	121 (74.2)	32 (74.4)	
Treatment modalities			0.03
Primary surgery ± adjuvant therapy	7 (16.3)	34 (20.7)	
Primary concurrent chemo-irradiation ± salvage surgery	21 (48.8)	42 (25.6)	
Primary radical RT ± salvage surgery or target therapy	9 (20.9)	37 (22.6)	
Neo-adjuvant chemotherapy + surgery or adjuvant therapy	4 (9.3)	9 (5.5)	
Other treatments	0 (0)	1 (0.6)	
Palliative treatments	1 (2.3)	28 (17.1)	
No treatment	1 (2.3)	13 (7.9)	
Primary curative treatment	*N = 41*	*N = 122*	0.17
Surgery-based	7 (17.1)	34 (27.9)	
RT-based	34 (82.9)	88 (72.1)	

Abbreviation: RT, radiotherapy.

Results

Subject recruitment

According to the Hong Kong Cancer Registry, 561 new cases of oropharyngeal cancer were recorded during the study period (2005–2009) of which 326 (58.1%) were SCCs and 167 (29.8%) were lymphoma. Among these OPSCC, 281 patients could be identified from records in public hospitals, and eventually 207 fulfilled our validation criteria. Hence, the final study population comprised 63.5% (207/326) of all OPSCC cases in Hong Kong diagnosed during the study period.

HPV status

All specimens provided adequate DNA quality for HPV detection by PCR using GP5+/GP6+_52HK primer. Altogether, 45 of 207 (21.7%) OPSCC specimens were positive for HPV DNA. Two of the 45 specimens did not yield RNA of sufficient quality for mRNA detection. The remaining 43 samples were all positive for E6*I mRNA. In order to ascertain the causative role of HPV, only cases positive for high-risk HPV E6*I mRNA were regarded as "HPV-associated OPSCC". Thus, the prevalence of HPV-associated OPSCCs was 20.8% (43/207). All these 43 specimens were HPV-16, except one HPV18.

Clinical features

Patients with HPV-associated OPSCC were significantly younger than those who were HPV-negative (mean age: 59.8 vs. 63.9 years, $P = 0.05$; proportion younger than 50 years: 23.3% vs. 10.4%, $P = 0.03$; Table 1). Overall, males accounted for the majority (86.0%) of OPSCC. However, HPV-associated OPSCC appeared to be more common among females (20.9 vs. 12.2%), although it did not reach statistical significance ($P = 0.15$). Multivariate analysis revealed that patients with HPV-associated OPSCC were more likely to be nonsmokers [39.5 vs. 15.1%, OR (95% CI): 2.89 (0.99–8.41), $P = 0.05$], nondrinkers [52.5 vs. 25.6%, OR (95% CI): 2.72 (1.04–7.13), $P = 0.04$], sited in the palatine tonsils [83.7 vs. 53.7%, OR (95% CI): 3.88 (1.36–11.05), $P = 0.01$], and present with an earlier primary tumor [T1 or T2; 79.1 vs. 47.6%, OR (95% CI): 3.81 (1.55–9.39), $P = 0.004$].

Patients with non–HPV-associated OPSCC were more likely to have a history of a previous head and neck cancer, most commonly (48.3%) of the nasopharynx, and have had previous irradiation. Although synchronous and metachronous tumors were more commonly found in the non-HPV group (0 vs. 7.3% and 2.3 vs. 7.4%, respectively), the differences did not reach statistical significance. Lung and esophagus were the two major sites for both synchronous and metachronous second primary tumors which accounted for more than half of the cases.

A distinct pattern of tumor staging at presentation was observed (Table 2). Non–HPV-associated OPSCC was more likely to present with an advanced primary tumor (T3 and T4) and node-negative disease. Moreover, metastatic disease was only detected in the non-HPV group, with lung and bone being the two major metastatic sites which accounted for more than three quarters of cases. Despite the absence of stage I disease in non–HPV-

Table 3. Association between histologic features and HPV status

	HPV-associated OPSCC ($N = 43$)/(%)	Non–HPV-associated OPSCC ($N = 164$)/(%)	Univariate analysis			Multivariate analysis[a]		
			OR	95% CI	*P*	OR	95% CI	*P*
Grade of differentiation					0.07			
Poorly	16 (37.2)	34 (20.7)	Ref.			Ref.		
Moderate	27 (62.8)	128 (78.0)	0.45	0.22–0.93	0.03	6.90	0.48–100.06	0.16
Well	0 (0)	2 (1.2)	<0.001	N.A.	1.00	127.86	N.A.	1.00
Basaloid differentiation[b]					<0.001			
No	28 (66.7)	152 (92.7)	Ref.			Ref.		
Yes	14 (33.3)	12 (7.3)	6.33	2.65–15.11	<0.001	19.74	2.34–166.15	0.006
Degree of keratinization					<0.001			
Absence/scanty	34 (79.1)	68 (41.5)	Ref.			Ref.		
Mild	8 (18.6)	50 (30.5)	0.32	0.14–0.75	0.009	0.27	0.07–1.13	0.07
Moderate	1 (2.3)	37 (22.6)	0.05	0.007–0.40	0.005	0.12	0.01–1.77	0.12
Severe	0 (0)	9 (5.5)	<0.001	N.A.	1.00	0.81	N.A.	1.00
Degree of necrotic region					0.03			
Absence	35 (81.4)	154 (93.9)	Ref.			Ref.		
Mild	6 (14.0)	6 (3.7)	4.40	1.34–14.46	0.02	1.20	0.22–6.43	0.83
Moderate	2 (4.7)	4 (2.4)	2.20	0.39–12.50	0.37	0.11	0.009–1.38	0.09
Nuclear/cytoplasm ratio					0.03			
High	26 (60.5)	62 (37.8)	Ref.			Ref.		
Moderate	17 (39.5)	101 (61.6)	0.40	0.20–0.80	0.009	0.42	N.A.	1.00
Low	0 (0)	1 (0.6)	<0.001	N.A.	1.00	0.18	N.A.	1.00
Degree of nuclear pleomorphism					0.12			
Severe	18 (41.9)	43 (26.2)	Ref.			Ref.		
Moderate	25 (58.1)	120 (73.2)	0.50	0.25–1.00	0.05	2.36	0.36–15.36	0.37
Mild	0 (0)	1 (0.6)	<0.001	N.A.	1.00	—		
p16 immunostaining					<0.001			
No	1 (2.3)	139 (84.8)	Ref.			Ref.		
Yes	42 (97.7)	25 (15.2)	233.52	30.72–1775.12	<0.001	4.316E+9	N.A.	1.00

[a]$R^2 = 76.1\%$.

[b]One case based on fine needle aspiration cytology was not included.

associated OPSCC, both groups had similar proportions of stage IV disease (Table 2).

Histologic and molecular features

HPV-associated OPSCC exhibited a distinct histologic profile compared with non–HPV-associated tumors (Table 3). It tended to be poorly differentiated, have a basaloid differentiation, have a more necrotic component, have absent or scanty keratinization and a higher nucleus to cytoplasm ratio. Multivariate analysis showed that basaloid differentiation was the only independent pathologic feature more commonly found in HPV-associated OPSCC [33.3 vs. 7.3%, OR (95% CI): 19.74 (2.34–166.15), $P = 0.006$].

All except one HPV-associated OPSCC specimens were positive for p16. Of the two cases with insufficient RNA quality for E6*I mRNA PCR, one was "p16-positive" and the other was "p-16 negative". p16 overexpression was strongly correlated with HPV status in OPSCC ($P < 0.001$). The sensitivity, specificity, positive and negative predictive values of p16 immunostaining for identification of HPV-associated OPSCC were 97.7% (42/43), 84.8% (139/164), 62.7% (42/67), 99.3% (139/140), respectively; whereas those for HPV DNA test using PCR based on consensus primers GP5+/GP6+_52HK were 100% (43/43), 98.8% (162/164), 95.6% (43/45), and 100% (162/162).

Outcomes

Patients with HPV-associated OPSCC have a significantly better 5-year OS and 5-year DSS compared with those with non–HPV-associated tumors [63.0% vs. 29.7%, HR (95% CI): 0.33 (0.20–0.56), $P < 0.001$ and 87.8% vs. 42.6%, HR (95% CI): 0.16 (0.07–0.40), $P < 0.001$; respectively; Fig. 1 and Supplementary Tables S1 and S2]. Features associated with a better OS included age <50 years, nonsmoker, p16 overexpression, early primary tumor (T1 or T2), nonmetastatic disease, and absence of synchronous tumor (Table 4). Better DSS was associated with nonsmoker, early primary tumors (T1 or T2), node-negative disease, nonmetastatic disease, p16 overexpression, and the absence of a synchronous tumor.

When a multivariate analysis was performed, only HPV-associated tumor, an earlier primary tumor (T1 or T2) and nonmetastatic disease were independent factors associated with a better OS; whereas HPV-associated tumors, nonsmokers, early primary disease, nonmetastatic disease, and the absence of synchronous tumors were independent factors associated with a better DSS (Table 4).

Recurrence after primary treatment was more common in non–HPV-associated OPSCC than in HPV-associated tumors, though the difference was not statistically significant (23.8% vs. 9.8%, $P = 0.07$; Table 5). Distant metastasis was the most common pattern of recurrence among patients with HPV-associated OPSCC, while these recurrences were similar in the non-HPV group. The lung was the most common site of a distant metastasis regardless of the HPV status in OPSCCs.

Discussion

Given the wide global variation in the reported prevalence of HPV-associated OPSCC which ranges from 0% to 86% (7, 20–25), it is necessary to have local population specific data to guide public health policy making. In this study, we used a stringent

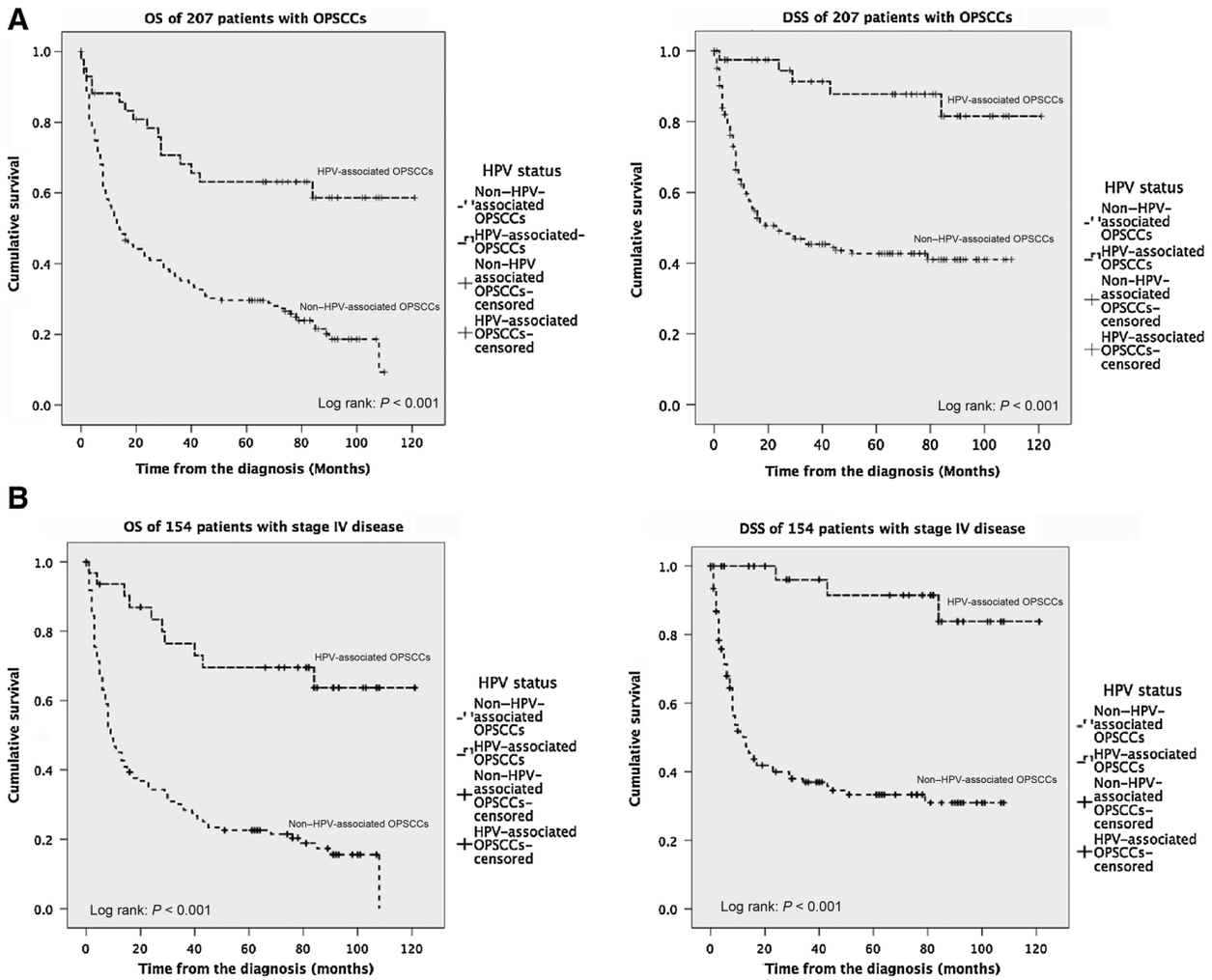

Figure 1.
Kaplan–Meier survival curve according to HPV status in (A) all patients and (B) patients with stage IV disease.

definition, the presence of E6/7 mRNA, to estimate the contribution of HPV in OPSCC in Hong Kong. With the coverage of the majority (63.5%) of cases that occurred in the whole territory during the 5-year study period, this study has generated the most representative information to date for Hong Kong, home to a southern Chinese population in a cosmopolitan city in east Asia. We found that HPV was attributed to 20.8% of OPSCC. Of note, the HPV-attributed portion for tonsil cancer was as high as 29.0%, which was higher than the 21% reported from a small-scale single-center study involving 49 cases collected over a longer period from 1985 to 2004 (10). Given the lack of good quality historical data for comparison, it is difficult to ascertain if there is a genuine change in the prevalence of HPV-associated OPSCC in Hong Kong. Nevertheless, given the recently reported increasing trend in many other countries and the only available local data aforementioned for comparison, it is necessary to start systematically monitoring the situation in Hong Kong by using the same definition of HPV-associated OPSCC. On the basis of data provided by the Hong Kong Cancer Registry, there were on average 65.2 new cases of OPSCC diagnosed in Hong Kong during our study period, corresponding to an age-standardized rate (ASR) of 0.60/100,000/year. Thus, we estimated that ASR of HPV-associated OPSCC would be 0.12/100,000/year during the 2005–2009 period.

Following the more stringent definition for HPV involvement, two cases that were positive for HPV DNA but with an insufficient RNA quality for E6*I mRNA analysis were excluded. One of them was HPV-16–positive, p16-positive, nonsmoking, nondrinking, and female. She had a T2N2bM0 tonsil SCC and was alive at her last follow-up at 98 months after the diagnosis. On the basis of these circumstantial features, her tumor was very likely to have been HPV associated. Thus, the portion of OPSCC attributed to HPV in Hong Kong may well be higher.

In line with the worldwide observation that HPV-16 is the predominant type of HPV found in oropharyngeal cancer (25–27), the vast majority (97.7%) of our HPV-positive cases were HPV-16. In view of the relatively higher prevalence of HPV52 in cervical cancer in east Asia (28, 29), and the recent report of finding several cases of HPV52 coinfections in OPSCC specimens collected from Beijing, China (30), we chose to use a PCR primer

Table 4. Association between clinical and pathologic factors with overall and disease-specific survival

	OS				DSS			
	Univariate analysis[a]		Multivariate analysis[b]		Univariate analysis[a]		Multivariate analysis[b]	
	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*
Age group								
≥50 years old	Ref.		Ref.		Ref.			
<50 years old	0.52 (0.29–0.93)	0.03	0.60 (0.32–1.12)	0.11	0.82 (0.44–1.55)	0.55		
Gender								
Male	Ref.				Ref.			
Female	0.60 (0.35–1.02)	0.06			0.56 (0.28–1.12)	0.10		
Smoking								
Ever smoker	Ref.		Ref.		Ref.		Ref.	
Never smoker	0.50 (0.31–0.81)	0.005	0.62 (0.38–1.02)	0.06	0.32 (0.15–0.65)	0.002	0.43 (0.20–0.92)	0.03
Drinking								
Ever drinker	Ref.				Ref.			
Never drinker	0.70 (0.48–1.03)	0.07			0.69 (0.42–1.13)	0.14		
HPV status								
Non–HPV–associated OPSCCs	Ref.		Ref.		Ref.		Ref.	
HPV-associated OPSCCs	0.33 (0.20–0.56)	<0.001	0.47 (0.23–0.94)	0.03	0.16 (0.07–0.40)	<0.001	0.19 (0.07–0.55)	0.002
p16 immunostaining								
Negative	Ref.		Ref.		Ref.		Ref.	
Positive	0.51 (0.35–0.75)	0.001	0.99 (0.59–1.67)	0.96	0.45 (0.27–0.75)	0.002	1.24 (0.67–2.28)	0.49
T stage								
Advanced (T3/T4)	Ref.		Ref.		Ref.		Ref.	
Early (T1/T2)	0.53 (0.38–0.74)	<0.001	0.54 (0.38–0.76)	<0.001	0.40 (0.26–0.61)	<0.001	0.39 (0.25–0.61)	<0.001
N stage								
Nodal positive (N1–3)	Ref.				Ref.		Ref.	
Nodal negative (N0)	0.85 (0.59–1.23)	0.39			0.59 (0.36–0.96)	0.04	0.60 (0.35–1.02)	0.06
Distant metastasis								
M1	Ref.		Ref.		Ref.		Ref.	
M0	0.19 (0.11–0.33)	<0.001	0.22 (0.12–0.38)	<0.001	0.14 (0.08–0.24)	<0.001	0.17 (0.09–0.30)	<0.001
Synchronous tumor								
Yes	Ref.		Ref.		Ref.		Ref.	
No	0.42 (0.23–0.76)	0.004	0.63 (0.34–1.15)	0.13	0.29 (0.16–0.54)	<0.001	0.49 (0.26–0.93)	0.03

[a]Univariate analysis: using log-rank test to assess the statistical significance.

[b]Multivariate analysis: Cox regression analysis (OS: −2 log likelihood: 1257.3 χ^2: 80.3 df: 7, *P*: <0.001; DSS: −2 Log likelihood: 783.7, χ^2: 115.3, df: 7, *P*: <0.001).

set that has been optimized for HPV-52 detection. However, we did not detect any HPV-52 from our OPSCC specimens. Nevertheless, the method we used only detects the predominant type from coinfections. Further investigations in other East Asian countries where HPV-52 is also found to be more prevalent in cervical cancer would be worthwhile.

We found that a history of head and neck cancer as well as metachronous and synchronous second primary cancers was more commonly found in non–HPV-associated OPSCC compared with the HPV-positive group, suggesting that different oncogenic mechanisms are involved. Of note, a recent Japanese study demonstrated a similar rate of second primary tumor among patients with HPV-positive (12.8%) and HPV-negative (14.6%) OPSCC (31). The predominant site of metachronous tumor in Japanese patients was the esophagus, whereas lung was the commonest site in our southern Chinese population. The underlying reasons for the discrepant observation need further investigation.

This study provides further evidence that HPV-associated OPSCC is a clinically distinctive disease, and the characteristic features observed in our southern Chinese patients concurs with those reported from the West, including a younger age, more likely to be nonsmokers and nondrinkers, a smaller primary tumor at presentation, and more advanced nodal metastases (32–35). We observed that there was a striking difference in the prognosis between HPV- and non–HPV-associated OPSCC presenting at an advanced stage. The 5-year OS and DSS were significantly better in HPV-associated cancer patients, regardless of the treatment modality. Besides the status of nonmetastatic disease, HPV positivity was the most important independent factor associated with the highest reduction in the risk of death for both OS and DSSs. The current AJCC cancer staging system, which does not consider the HPV status, is therefore inadequate in predicting the prognosis of HPV-associated OPSCC. Incorporating the HPV status into the current staging system is necessary to achieve a better clinical stratification.

Given the superior prognosis of HPV-associated OPSCC regardless of the modality of treatment, and the improved operative safety of newer surgical techniques, the focus of primary treatment in OPSCC has shifted to a surgical management. In the recent decade, attempts using transoral robotic surgery (TORS) for OPSCC have shown promising outcomes, reaching up to 98% and 90% DSS at a 1-year and 2-year follow-up, respectively (36, 37). As shown in this study, primary curative chemoirradiation is the predominant treatment modality (75%) for OPSCC in Hong Kong. Given the likelihood of an increase in the incidence of HPV-associated OPSCC in Asia, local studies are needed to verify the long-term efficacy of upfront surgical treatment or other de-escalating treatment regimens. With various ongoing randomized clinical trials in the West, especially those on assessing the efficacy of upfront surgery instead of primary chemoirradiation (NCT01898494-ECOG3311; NCT02072148-SIRS trial, https://clinicaltrials.gov), there is likely to be a shift in the treatment paradigm for HPV-associated OPSCC in the near future.

Table 5. Association between clinical outcome and HPV status

	HPV-associated OPSCC[a] *N* (%)	Non–HPV-associated OPSCC[a] *N* (%)	*P*
Metachronous tumors	*N* = 43	*N* = 164	0.31
Yes	1 (2.3)	12 (7.3)	
No	42 (97.7)	152 (92.7)	
Residual disease after curative treatment			0.009
Yes	2 (4.9)	28 (23.0)	
No	39 (95.1)	94 (77.0)	
Recurrence after curative treatment			0.07
Yes	4 (9.8)	29 (23.8)	
No	37 (90.2)	93 (76.2)	
Types of recurrence after curative treatment	*N* = 4	*N* = 29	0.32
Local	0 (0)	7 (24.1)	
Regional	0 (0)	6 (20.7)	
Distant	2 (50.0)	8 (27.6)	
Loco-regional	0 (0)	4 (13.8)	
Local + distant metastasis	0 (0)	1 (3.4)	
Regional + distant metastasis	1 (25.0)	2 (6.9)	
Loco-regional + distant metastasis	1 (25.0)	1 (3.4)	
Local recurrence			0.19
Yes	1 (2.4)	13 (10.7)	
No	40 (97.6)	108 (89.3)	
Regional recurrence			0.36
Yes	2 (4.9)	13 (10.7)	
No	39 (95.1)	109 (89.3)	
Distant metastasis			1.00
Yes	4 (9.8)	12 (9.8)	
No	37 (90.2)	110 (90.2)	

[a]Only patients who received primary curative treatment were included in the analysis; *N* = 41 for HPV-associated OPSCC; *N* = 122 for non–HPV-associated OPSCC.

Conclusion

This large-scale multi-institutional study has provided the best available estimate on the incidence of HPV-associated OPSCC in Hong Kong, which is instrumental for monitoring the future trend of change. If our population follows the global trend, we should anticipate and prepare for an increase in HPV-related OPSCC. Further studies to explore the potential of using HPV-based methods to screen for and to classify the stages of OPSCC are essential. Evaluating the long-term efficacy of using de-escalating treatment modalities for HPV-associated OPSCC is warranted.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Acknowledgments

The authors thank all pathology laboratory personnel, especially Stephen Lo, Wendy Ho, Apple CM Yeung, and Dr. Joanna Tong for their endless support in various laboratory tests. The verification of the patients' identity and the analysis of age-standardized rate of OPSCC conducted by Oscar Mang from the Hong Kong Cancer Registry is highly appreciated. We also thank Dr. Stephen W.Y. Lau from Department of ENT of Queen Elizabeth Hospital and all collaborators from various Departments of ENT, Pathology, and Oncology for their contribution in this study.

Grant Support

This work was supported by the Health and Medical Research Fund (HMRF), Food and Health Bureau, the Government of the Hong Kong Special Administrative Region (Ref No: 11122911). E.W.H. Lam, J.Y.W. Chan, C.S. Ng, S.T.H. Lo, V.S.C. Lam, A.C. Vlantis, and R.K.H. Ma had been awarded a grant from the Health and Medical Research Fund (HMRF), Food and Health Bureau, the Government of the Hong Kong Special Administrative Region (Project no: 11122911) to conduct this project.

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Received August 12, 2015; revised October 7, 2015; accepted October 8, 2015; published OnlineFirst November 24, 2015.

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