Smoking, human papillomavirus infection, and p53 mutation as risk factors in oropharyngeal cancer: a case-control study

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KEY MESSAGES

1. In Hong Kong, infection with high-risk human papillomavirus (HPV) over the head and neck mucosa is not uncommon.
2. The association between HPV and head and neck cancer is site-specific, and mainly confined to the oropharynx.
3. About 26% to 30% of oropharyngeal carcinoma is associated with high-risk HPV infection, mostly HPV16. Smoking that predisposes to TP53 mutation is another risk factor.
4. There is a potential to use HPV-based non-invasive methods to screen for early oropharyngeal carcinoma. Early detection of HPV-associated cancer is associated with better response to treatment and should be a public health priority.

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Introduction

Worldwide, more than 550 000 cases of head and neck squamous cell carcinoma (HNSCC) are reported annually. Infection with human papillomavirus (HPV) plays an aetiological role in a proportion of HNSCC cases, particularly those with involvement of the palatine and lingual tonsils. This study aimed to delineate the role of HPV in HNSCC in Hong Kong.

Methods

This cross-sectional case-control study was conducted from January 2012 to December 2014 and was approved by the ethics committee of the participating public hospitals. Patients with suspected benign or malignant lesions over the head and neck mucosa were recruited. Patients with nasopharyngeal carcinoma, known recurrent cancers, or metastasis from a primary site outside the head and neck region were excluded.

HPV DNA was detected by nested polymerase chain reaction and typed by sequencing. Expression of viral oncoproteins E6/E7 was examined by measuring E6*I mRNA to define the oncogenic role. Viral integration was determined by comparing the number of copies of E2 and E7 genes.[1]

Exons 4-9 of TP53 were amplified by polymerase chain reaction and sequencing. Mutations were determined based on the reference sequences available at the International Agency for Research on Cancer TP53 database (http://p53.iarc.fr).

Results

Of the 256 subjects with histologically confirmed malignant lesions, 228 (89.1%) were HNSCC, 13 (5.1%) were salivary gland malignant tumours, and 15 (5.9%) were cancers of other histological types (Table 1). In addition, 283 controls with benign or inflammatory lesions or normal tissue collected over the head and neck mucosa were divided into different groups according to the anatomic sites where tissue samples were taken (Tables 2 and 3).

Smoking

Compared with controls, subjects with malignant lesions were more likely to have a history of smoking (48.4% vs 31.4%, P<0.001, Table 1). Subjects with SCC of the oropharynx, larynx, and hypopharynx had a higher prevalence of smoking history than those with SCC of the oral cavity, lip and paranasal sinus, and those with salivary gland malignant tumour and cancer of other histological types (67.6-80.6% vs 20.0-46.2%, Table 1). Compared with controls with lesions at the same anatomic site,

TABLE 1. Prevalence of human papillomavirus (HPV) infection, TP53 mutation, and smoking status

Disease status	Male:female ratio	Mean±SD (range) age (years)	No. (%) of subjects		
			HPV DNA positive	TP53 mutation	Ever smoked
Malignant cases of the head and neck region (n=256)	2.1:1	62.7±13.5 (21-92)	27 (10.5)	52 (20.3)	124 (48.4)
Head and neck squamous cell carcinoma (SCC) [n=228]	2.1:1	62.6±13.3 (27-92)	22 (9.6)	47 (20.6)	112 (49.1)
Oral cavity SCC (n=137)	1.2:1	63.5±14.3 (27-92)	3 (2.2)	26 (19.0)	47 (34.3)
Oropharyngeal SCC (n=34)	7.5:1	56.4±13.6 (30-88)	10 (29.4)	8 (23.5)	23 (67.6)
Laryngeal SCC (n=31)	All male	64.8±9.0 (48-84)	5 (16.1)	7 (22.6)	25 (80.6)
Hypopharyngeal SCC (n=21)	4.3:1	63.0±9.4 (49-83)	3 (14.3)	4 (19.0)	16 (76.2)
Lip and paranasal sinus SCC (n=5)	0.3:1	65.2±14.5 (46-82)	1 (20.0)	2 (40.0)	1 (20.0)
Salivary gland malignant tumours (n=13)	3.3:1	61.2±13.2 (38-84)	2 (15.4)	2 (15.4)	6 (46.2)
Cancer of other histological types (n=15)	2.0:1	64.3±15.9 (21-86)	3 (20.0)	3 (20.0)	6 (40.0)
Non-malignant controls (n=283)	1.1:1	57.0±14.4 (20-91)	30 (10.6)	16 (5.7)	89 (31.4)
Oral cavity (n=139)	4.0:1	59.7±13.9 (21-91)	16 (11.5)	3 (2.2)	35 (25.2)
Oropharynx (n=42)	1.5:1	50.4±15.5 (24-81)	2 (4.8)	4 (9.5)	17 (40.5)
Larynx (n=34)	5.8:1	54.6±13.7 (28-76)	6 (17.6)	4 (11.8)	18 (52.9)
Lip and paranasal sinus (n=20)	0.7:1	62.4±10.1 (46-84)	3 (15.0)	3 (15.0)	5 (25.0)
Salivary gland (n=48)	1.2:1	54.8±14.7 (20-81)	3 (6.3)	2 (4.2)	14 (29.2)

TABLE 2. TP53 mutation status among subjects with and without malignant tumour

Disease status	No. (%) of subjects		No. (%) of subjects with any TP53 mutation								
	Any mutation	Any non-synonymous mutation	Any trans-version	Any tran-sition	Non-CpG trans-version	A:T > T:A	A:T > C:G	A:T > G:C	G:C > A:T	G:C > C:G	G:C > T:A
Head and neck cancer overall (n=256)	52 (20.3)	43 (16.8)	35 (67.3)	17 (32.7)	25 (48.1)	7 (13.5)	1 (1.9)	6 (11.5)	11 (21.2)	4 (7.7)	23 (44.2)
Control group 1 (all) [n=283]	16 (5.7)	15 (5.3)	9 (56.3)	7 (43.8)	9 (56.3)	4 (25.0)	0 (0.0)	2 (12.5)	5 (31.3)	0 (0.0)	5 (31.3)
P value	<0.001	<0.001									
Head and neck squamous cell carcinoma (n=288)	47 (20.6)	40 (17.5)	32 (68.1)	15 (31.9)	22 (46.8)	5 (10.6)	1 (2.1)	6 (12.8)	9 (19.1)	4 (8.5)	22 (46.8)
Control group 2 (all except salivary gland) [n=235]	14 (6.0)	13 (5.5)	8 (57.1)	6 (42.9)	8 (57.1)	3 (21.4)	0 (0.0)	1 (7.1)	5 (35.7)	0 (0.0)	5 (35.7)
P value	<0.001	<0.001									
Oral cavity squamous cell carcinoma (n=137)	26 (19.0)	22 (16.1)	16 (61.5)	10 (38.5)	12 (46.2)	1 (3.8)	1 (3.8)	3 (11.5)	7 (26.9)	2 (7.7)	12 (46.2)
Control group 3 (oral cavity) [n=139]	3 (2.2)	3 (2.2)	1 (33.3)	2 (66.7)	1 (33.3)	0 (0.0)	0 (0.0)	0 (0.0)	2 (66.7)	0 (0.0)	0 (0.0)
P value	<0.001	<0.001									
Oropharyngeal squamous cell carcinoma (n=34)	8 (23.5)	7 (20.6)	4 (50.0)	4 (50.0)	3 (37.5)	1 (12.5)	0 (0.0)	3 (37.5)	1 (12.5)	0 (0.0)	3 (37.5)
Control group 4 (oropharynx) [n=42]	4 (9.5)	4 (9.5)	3 (75.0)	1 (25.0)	3 (75.0)	1 (25.0)	0 (0.0)	0 (0.0)	1 (25.0)	0 (0.0)	2 (50.0)
P value	0.177	0.204									
Laryngeal squamous cell carcinoma (n=31)	7 (22.6)	5 (16.1)	6 (85.7)	1 (14.3)	3 (42.9)	1 (14.3)	0 (0.0)	0 (0.0)	1 (14.3)	1 (14.3)	4 (57.1)
Control group 5 (larynx) [n=34]	4 (11.8)	3 (8.8)	3 (75.0)	1 (25.0)	3 (75.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (25.0)	0 (0.0)	3 (75.0)
P value	0.406	0.463									
Head and neck cancer overall + control groups 1-5 (n=539)	**68 (12.6)**	**58 (10.8)**	**44 (64.7)**	**24 (35.3)**	**34 (50.0)**	**11 (16.2)**	**1 (1.5)**	**8 (11.8)**	**16 (23.5)**	**4 (5.9)**	**28 (41.2)**

TABLE 3. Human papillomavirus (HPV) infection status among subjects with and without malignant tumour

Disease status	No. (%) of subjects				
	HPV DNA (any type) positive	HPV DNA (high-risk types of HPV16, 31, and 52) positive	HPV16 DNA positive	HPV integration positive*	HPV E6*I mRNA positive*
Head and neck cancer overall (n=256)	27 (10.5)	22 (8.6)	20 (7.8)	14 (5.5)	11 (4.3)
Control group 1 (all) [n=283]	30 (10.6)	24 (5.8)	22 (7.8)	2 (0.7)	1 (0.4)
Head and neck squamous cell carcinoma (n=228)	22 (9.6)	17 (7.5)	16 (7.0)	12 (5.3)	10 (4.4)
Control group 2 (all except salivary gland) [n=235]	26 (11.1)	21 (8.9)	19 (8.1)	2 (0.9)	1 (0.4)
Oral cavity squamous cell carcinoma (n=137)	3 (2.2)	1 (0.7)	1 (0.7)	1 (0.7)	0 (0.0)
Control group 3 (oral cavity) [n=139]	16 (11.5)	15 (10.8)	13 (9.4)	1 (0.7)	1 (0.7)
Oropharyngeal squamous cell carcinoma (n=34)	10 (29.4)	10 (29.4)	10 (29.4)	10 (29.4)	9 (26.5)
Control group 4 (oropharynx) [n=42]	2 (4.8)	2 (4.8)	2 (4.8)	0 (0.0)	0 (0.0)
Laryngeal squamous cell carcinoma (n=31)	5 (16.1)	3 (9.7)	3 (9.7)	1 (3.2)	1 (3.2)
Control group 5 (larynx) [n=34]	5 (14.7)	1 (2.9)	1 (2.9)	0 (0.0)	0 (0.0)
Head and neck cancer overall + control groups 1-5 (n=539)	**56 (10.4)**	**46 (8.5)**	**42 (7.8)**	**16 (3.0)**	**12 (2.2)**

* HPV E6*I mRNA detection was performed for high-risk HPV types only. All samples positive for HPV E6*I mRNA were positive for integration

subjects with oropharyngeal and laryngeal SCC had a higher prevalence of self-reported history of smoking (P=0.018 for both).

TP53 mutation

A total of 40 different mutations of TP53 were found; nine located at hot-spots. Of the 40 mutations, 36 (90.0%) were non-synonymous and resulted in amino acid substitution, and five (12.5%) resulted in a stop codon. The most frequently found mutation was located at exon 4 resulting in change of codon 37 from serine to threonine (n=9), followed by mutation at codon 249 from arginine to methionine (n=8) and mutation at codon 176 from cysteine to phenylalanine (n=6). Of 68 subjects with TP53 mutations, 66 (97.1%) had mutation at one spot and two had mutations at two spots who were ex-smokers and one had laryngeal SCC and the other had oral cavity SCC.

The prevalence of TP53 mutation was higher in subjects with head and neck cancer overall (20.3% vs 5.7%, P<0.001), HNSCC (20.6% vs 6.0%, P<0.001), and oral cavity SCC (19.0% vs 2.2%, P<0.001), compared with the corresponding control groups (Table 2). Transversion mutations were about twice as common as transition mutations in terms of head and neck cancer overall (67.3% vs 32.7%), HNSCC (68.1% vs 31.9%), and oral cavity SCC (61.5% vs 38.5%). Furthermore, 77.3% of transversion mutations occurred in non-CpG sites, and G:C > T:A was the most frequent pattern of substitution. Polymorphisms (Arg/Arg, Arg/Pro, Pro/Pro) at codon 72 were detected at similar frequencies; the cancer groups did not differ significantly to their corresponding control group.

Human papillomavirus infection

HPV DNA (all types) was found in 56 (10.4%) samples, with HPV16 the most common (n=42, 75.0%), followed by HPV6 (n=7, 12.5%), HPV11 (n=3, 5.4%), HPV31 (n=2, 3.6%), and HPV52 (n=2, 3.6%) [Table 3]. Only 16 (38.1%) of the 42 HPV16-positive samples showed integration based on the comparison between gene copies of E2 and E7. The two HPV31-positive and the two HPV52-postive samples were negative for integration. Furthermore, 12 (75.0%) of 16 samples that showed HPV16 integration were positive for HPV E6*I mRNA (Table 3).

Cases of oropharyngeal SCC showed the highest rate for high-risk HPV DNA and differed significantly to the corresponding control group (29.4% vs. 4.8%, P=0.003, Table 3). All HPV-positive oropharyngeal SCC samples were HPV16 integration positive, and in nine out of ten cases, HPV16 E*I mRNA was also detected.

One of the five high-risk HPVs identified from laryngeal SCC specimens was positive for viral integration and E6*I mRNA expression. The overall HPV DNA positive rate was 10.4% for the control groups, and was as high as 14.7% for laryngeal samples (Table 3). Of note, one specimen from the oral cavity and another specimen from the larynx in the control group were positive for HPV16 integration and E6*I mRNA.

Sexual and drinking history

A history of sexually transmitted disease was reported in 6.7% of subjects, and was significantly more common among head and neck cancer overall (9.8% vs 3.9%, P=0.006), HNSCC (10.5% vs 4.7%, P=0.017), and oropharyngeal SCC (20.6% vs 0%, P=0.002). Only 15 (2.8%) subjects reported a history of HPV disease, mainly genital warts (80%); no significant difference between subject groups was observed. Altogether, 13.0% of subjects reported no sexual partner over their lifetime; no significant difference across different groups was observed. Compared with their respective control group, patients with oropharyngeal SCC were less likely to report having just one sexual partner over their lifetime (35.3% vs 66.7%, P=0.006), whereas patients with laryngeal SCC were less likely to report having oral sex (12.9% vs 35.3%, P=0.046).

Patients with head and neck cancer overall, HNSCC, and oral cavity SCC were more likely to report a history of regular drinking than their corresponding control groups. Similar associations were obtained when the analysis was focused on heavy drinking (≥5 glasses of beer/wine or >3 glasses of cocktail per day).

Multivariate analysis

Variables with a significant (P<0.05) or close to significant (P<0.1) association with HNSCC or oropharyngeal SCC in univariate analysis were assessed using a logistic regression model. Independent risk factors for HNSCC were older age (odds ratio [OR]=1.03, 95% confidence interval [CI]=1.02-1.05 per year older), TP53 mutation (OR=3.38, 95% CI=1.71-6.66), and HPV16 infection with oncogenic phenotype (integration and E6/7 mRNA expression) [OR=9.19, 95% CI=1.13-74.68). Independent risk factors for oropharyngeal SCC were male gender (OR=4.44, 95% CI=1.45-17.28), older age (OR=1.05, 95% CI=1.01-1.09 per year older), and having ≥1 sexual partner in a lifetime (OR=4.10, 95% CI=1.35-12.42). High-risk HPV infection was an effect modifier of the association between smoking and HNSCC. Among high-risk HPV infection positive subjects, non-smokers were less likely to have HNSCC than smokers (OR=0.03, 95% CI=0.00-0.23, P<0.005). Compared with high-risk HPV infection positive ever-smokers, high-risk HPV negative ever-smokers (OR=0.29, 95% CI=0.08-1.06, P=0.06) and non-smokers (OR=0.28, 95% CI=0.07-1.06, P=0.06) were less likely to have HNSCC. An interaction between high-risk HPV infection and TP53 mutation on the association with HNSCC was suggested. Among subjects negative for high-risk HPV infection, TP53 mutation was more likely to associate with HNSCC compared with those without TP53 mutation (OR=3.35, 95% CI=1.61-7.00, P<0.005). In subjects positive for high-risk HPV, no significant difference was observed between the groups with and without TP53 mutation for the association with HNSCC.

Discussion

HPV plays an aetiological role in oropharyngeal SCC, especially those involving the palatine and lingual tonsils. The proportion of these tumours attributed to HPV varies widely across the world, and information on Southern Chinese is limited. An aetiological association with carcinoma that develops at sites other than the oropharynx is not well known.

In the current study, about 10% of samples were HPV positive of which >80% were high-risk types. Of note, less than one third of these high-risk HPV types demonstrated evidence of viral integration or oncogene E6/7 mRNA expression. Studies that only identify HPV down to the type level without verifying the viral integration or E6/7 mRNA expression status may have overestimated the role of HPV in head and neck cancer.

By taking either E6/7 mRNA expression or viral integration as an indication of oncogenic phenotype of HPV infection, we estimated that close to 30% of oropharyngeal SCC in Hong Kong may be associated with HPV. This attributed fraction is lower than that reported from Japan (50%)[2] or Beijing (40%),[3] but is close to that from Korea (32%).[4]

A meta-analysis concluded that there was a high prevalence (30%) of HPV16/18 in laryngeal cancer specimens collected from Chinese patients with an OR of 8.07.[5] In our series, 9.7% of patients with laryngeal SCC had HPV16 infection, but only one third had evidence of integration or E6/7 mRNA expression. More in-depth studies to scrutinise the role of HPV in laryngeal cancer are needed.

High-risk HPV types encode E6 protein which disrupts the normal function of p53 and thus escapes the need for TP53 mutation in cancer development. In our study, 20.3% of subjects with HNSCC were positive for TP53 mutation. The mutation rate was lower than that reported from Europe and America, but was comparable with that from Asia. The most common pattern of mutation was G:C > T:A transversion, which is known to associate with tobacco exposure. We also observed a positive association between TP53 mutation and smoking.

Conclusions

In Hong Kong, high-risk HPV infection and TP53 mutation are independent risk factors for HNSCC. The association with high-risk HPV infection was site-specific and mainly confined to the oropharynx where palatine and lingual tonsillar carcinoma develop. About 26% to 30% of oropharyngeal carcinoma may be associated with high-risk HPV

infection, mostly HPV16. Smoking that predisposes to TP53 mutation is another risk factor. High-risk HPV infection enhances the effect of smoking on HNSCC development. Subjects positive for high-risk HPV infection were less dependent on TP53 mutation as a risk factor for HNSCC.

There is a potential to use HPV-based non-invasive methods to screen for early oropharyngeal carcinoma. Early detection of HPV-associated cancer is associated with a better response to treatment and should be a public health priority.

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