

Human papillomavirus status in southern Chinese women

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KEY MESSAGES

1. The overall and type-specific human papillomavirus (HPV) prevalence differed between Hong Kong and Guangzhou healthy women. The prevalence of HPV was significantly higher in Guangzhou than Hong Kong women. Younger women had significantly higher risk of HPV infection.
2. HPV16 remained the most common type detected in both regions; the frequency increased with increasing disease severity. The prevalence of HPV58 and HPV52 was relatively high in women with normal cervix and precancerous lesions.

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Introduction

Human papillomavirus (HPV) is a sexually transmitted pathogen that plays an important role in the pathogenesis of precancerous cervical lesions and cervical cancer.¹ The prevalence of HPV types in Hong Kong differs from that in Sichuan, China. Cross-border travel and marriage between people in Hong Kong and Guangzhou have increased greatly, as has the potential for disease transmission.

Integration of HPV correlates with poor response to treatment and poor disease-free survival in cervical cancer.² Integration usually causes deletion and/or disruption of the E2 gene of the HPV. In this study, we aimed to determine (1) the spectrum and prevalence of HPV in healthy women in Hong Kong and Guangzhou, and (2) whether integration of the high-risk HPV16 and HPV58 genomes in the host is associated with progressive severity of the precancerous cervical lesion and cervical cancer.

Methods

This cross-sectional study was approved by the local institutional review board and conducted from October 2006 to September 2008, in collaboration with the Department of Obstetrics and Gynaecology and Department of Pathology of The University of Hong Kong, the Family Planning Association of Hong Kong, and the Department of Obstetrics and Gynaecology of the Second Affiliated Hospital, Sun Yet-Sen University, Guangzhou, Guangdong, China.

The prevalence of HPV and type-specific infections in healthy women and women with precancerous lesions and cervical cancer were

compared between Hong Kong and Guangzhou. The association between co-factors and the risk of HPV infection was analysed. The integrations of HPV16 and HPV58 viral DNA in host genomes were assessed and the clinical significance was determined.

Cytology samples of 1280 and 1273 healthy women in Hong Kong and Guangzhou were collected, respectively. The exfoliated cervical cells were collected by ThinPrep (Hologic, Bedford, MA, USA) in Hong Kong or liquid-based cervical cytology test in Guangzhou. In addition, 438 and 204 samples of cervical precancerous lesions and cervical cancers were retrospectively selected in the two regions, respectively.

A Hybribio DNA extraction kit (Hybribio, Hong Kong) and QIAamp DNA FFPE Tissue Kit (Qiagen, Hilden, Germany) were used for DNA extraction from the cytological remnants and the paraffin-embedded tissues, respectively. Genotyping of HPV was performed using the GenoArray HPV genotyping test (Hybribio), which is a polymerase chain reaction (PCR)-based assay that is capable of amplifying 21 HPV genotypes, including 13 high-risk types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) and nine other-risk types.

The HPV viral DNA integration was determined by quantitative real-time fluorescent PCR with five sets of E2 and one set of E6 primers for HPV16 and HPV58. The proportion of integrated value was calculated by the ratio of E2 value (episomal) to E6 value (episomal and integrated).

Results

Of the 1280 and 1273 cytology samples from Hong

TABLE I. Comparison of the prevalence of human papillomavirus (HPV) infections in Hong Kong and Guangzhou women

HPV prevalence	Hong Kong (% positive)				Guangzhou (% positive)			
	Normal (age- adjusted)	Low-grade cervical intraepithelial neoplasia	High-grade cervical intraepithelial neoplasia	Cervical cancer	Normal (age- adjusted)	Low-grade cervical intraepithelial neoplasia	High-grade cervical intraepithelial neoplasia	Cervical cancer
Overall prevalence	5.6‡	22.4‡	30.8	40.2	10.1‡	6.0‡	28.9	39.2
Multiple infection	1.8	1.9	0.9	1.0	1.8	0	1.8	2.0
Multiple infection among HPV-positive	33.7†	8.3	2.8	2.4	15.3†	0	6.1	5.0
High-risk HPV	4.0‡	31.0†	28.2	40.2	7.6‡	5.0†	23.7	39.2
High-risk types among HPV-positive	71.9	79.2	91.7	100	75.5	83.3	81.8	100
Type-specific (% HPV-positive)								
HPV16	23.6*	16.7	27.8	56.1*	11.0*	16.7	36.4	77.5*
HPV18	14.6*	12.5	0*	22.0	4.9*	0	15.2*	17.5
HPV31	0‡	0	13.9*	4.9	16.6‡	33.3	0*	0
HPV52	19.1	20.8	13.9	7.3	23.9	33.3	9.1	0
HPV58	20.2*	29.2†	27.8	9.8	10.4*	0†	12.1	5.0
Total (5 types)	77.5	79.2	83.4	100	66.8	83.3	72.8	100

* P=0.01 to <0.05, Chi-squared test

† P=0.001 to <0.01, Chi-squared test

‡ P<0.001, Chi-squared test

Kong and Guangzhou women, respectively, 1245 (97.3%) and 1209 (95.0%) had normal cytology. The age-adjusted HPV prevalence was significantly lower in Hong Kong than Guangzhou women (5.6% [89/1245] vs 10.1% [163/1209], Table 1), particularly in the three younger age-groups (Fig). The prevalence of HPV infection varied between age-groups in the two regions.

In Hong Kong, the HPV prevalences in women with low-grade cervical intraepithelial neoplasia (LG CIN), high-grade CIN (HG CIN), and cervical cancer were 22.4%, 30.8%, and 40.2%, respectively. In Guangzhou, the corresponding prevalences were 6.0%, 28.9%, and 39.2%, respectively. The HPV prevalence in women with LG CIN was significantly higher in Hong Kong than Guangzhou ($P<0.001$). The rate for age-adjusted multiple HPV infection was 1.8% in women with normal cervix for both regions, accounting for 33.7% and 15.3% of HPV-positive cases in Hong Kong and Guangzhou cohorts, respectively ($P=0.001$).

In Hong Kong, HPV16 was most commonly identified in women with normal cytology, HG CIN, and cervical cancer, whereas HPV58 was most commonly identified in women with LG CIN. In Guangzhou, HPV16 was most commonly identified in women with HG CIN and cervical cancer, whereas HPV52 and HPV31 were most commonly identified in women with normal cytology and LG CIN. HPV16, 52 and 58 were the most common high-risk types detected in women with normal cytology in both regions. The frequency of HPV16 in women with normal cervix and cervical cancer was significantly higher in Hong Kong than in Guangzhou ($P=0.015$ and $P=0.036$, respectively). The frequency of HPV16 increased with an increase of disease severity. The

frequencies of HPV58 and 52 were higher in women with normal cervix and precancerous lesion in both regions.

Age was a risk factor only in Hong Kong women younger than 29 years, compared with other age-groups ($P=0.009$). Previous Pap smear test was another risk factor for women in Hong Kong ($P=0.037$). Women with a previous abnormal Pap smear test also had significantly higher HPV infection ($P=0.021$). In Guangzhou, women with previous abnormal cytological findings also had increased risk

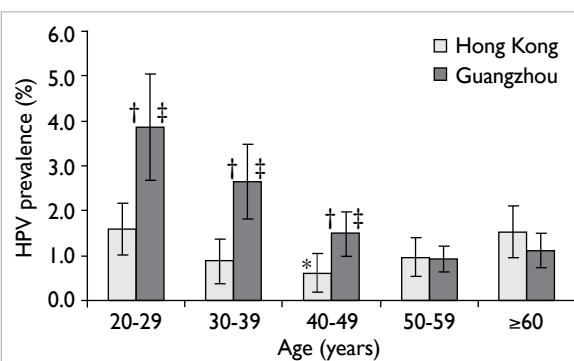


FIG. Age-adjusted human papillomavirus (HPV) infection prevalences in five age-groups in Hong Kong and Guangzhou women.

* In Hong Kong, the prevalence of HPV infection is significantly lower in the age-group of 40-49 years than that of 20-29 years and ≥60 years ($P<0.05$)

† The prevalence of HPV infection is significantly higher in Guangzhou than Hong Kong women in the age-groups of 20-29 years ($P<0.001$), 30-39 years ($P<0.001$), and 40-49 years ($P=0.009$)

‡ In Guangzhou, the prevalence of HPV infection is significantly higher in the age-groups of 20-29 years, 30-39 years, and 40-49 years than in the other groups ($P<0.05$)

of HPV infection ($P=0.044$). Two sexual behaviour variables (lifetime number of sexual partners and age at first sexual intercourse) were associated with the risk of HPV infection. In Guangzhou, the risk of HPV infection was significantly higher in women with ≥ 3 than 1-2 lifetime sexual partners ($P<0.001$). In Hong Kong, women having first sexual intercourse before the age of 20 years had higher risk than those having first sexual intercourse after the age of 21 years ($P=0.043$). After multivariable analysis, only the lifetime number of sexual partners remained a significant factor in Guangzhou women (Table 2). No association was noted with variables of gravidity, use of condom or oral contraceptive, history of sexually transmitted diseases, and smoking.

The complete episomal form was found in

only 3/110 (2.7%) HPV16- and 4/48 (8.3%) HPV58-positive samples. HPV16 integration and HPV58 integration were detected in 107 and 44 samples, respectively; 30 (27.3%) HPV16-positive samples and only one (2.1%) HPV58-positive sample showed complete integration. No significant difference in the integration patterns of HPV16 or HPV58 with regard to the various grades of cervical lesions was noted. The distribution and frequency of HPV16 or HPV58 DNA integration were similar between the Hong Kong and Guangzhou regions.

Discussion

In Guangzhou, the age-adjusted HPV infection rate was 10.1% in women with normal cytology, which is

TABLE 2. Multiple logistic regression for risk factors of human papillomavirus (HPV) infection in Hong Kong and Guangzhou women

Variable	Hong Kong				Guangzhou			
	No.	HPV-positive (%)	OR (95% CI)	P value	No.	HPV-positive (%)	OR (95% CI)	P value
Age (years)								
20-29	248	10.9	1	0.025	233	15.0	1	0.626
30-39	243	4.9	0.53 (0.24-1.15)		237	14.3	1.48 (0.79-2.77)	
40-49	249	3.2	0.37 (0.14-1.00)		236	12.7	1.19 (0.61-2.33)	
50-59	250	7.2	0.77 (0.29-2.01)		253	13.8	1.55 (0.77-3.10)	
≥ 60	255	9.4	1.32 (0.50-3.48)		250	11.6	1.21 (0.59-2.49)	
Previous Pap smear test								
Yes	1040	6.4	1	0.038	377	11.4	1	0.087
No	205	10.7	0.49 (0.25-0.96)		832	14.4	0.71 (0.48-1.05)	
Lifetime number of sexual partner								
1	868	6.8	1	0.973	1039	12.4	1	0.015
2	185	7.0	0.92 (0.45-1.88)		123	13.8	1.21 (0.67-2.18)	
≥ 3	192	8.9	0.98 (0.46-2.09)		46	34.8	3.20 (1.45-7.04)	
Age at first sexual intercourse (years)								
≤ 20	430	17.8	1	0.236	176	14.2	1	0.701
21-25	510	5.3	0.55 (0.30-1.00)		643	14.3	1.31 (0.76-2.26)	
26-35	292	6.2	0.62 (0.30-1.28)		385	11.7	1.11 (0.58-2.10)	
≥ 36	13	7.7	1.14 (0.13-10.26)		5	20.0	1.64 (0.17-16.20)	
Sexually transmitted disease history								
Never	1214	7.2	1	0.95	1170	13.2	1	0.368
Ever	16	6.3	0.94 (0.12-7.52)		17	29.4	1.74 (0.52-5.76)	
Oral contraceptive use								
Never	551	6.5	1	0.197	1031	13.2	1	0.588
Ever	683	7.6	1.38 (0.85-2.26)		177	14.6	1.14 (0.71-1.84)	
Condom use								
Never or rare	672	8.3	1	0.152	877	13.7	1	0.888
Regular	549	5.6	0.69 (0.42-1.15)		318	12.9	0.97 (0.64-1.47)	
Gravidity								
0	354	6.5	1	0.065	86	20.9	1	0.348
1-2	455	8.4	1.87 (0.92-3.80)		548	12.4	0.55 (2.80-1.08)	
3-4	334	7.2	1.25 (0.52-3.00)		455	13.6	0.52 (0.24-1.09)	
≥ 5	100	4.0	0.55 (0.45-2.04)		120	12.5	0.52 (0.21-1.28)	
Smoking								
Never	1105	6.7	1	0.808	1173	13.3	1	0.958
Former	72	8.3	1.09 (0.43-2.77)		17	17.6	1.22 (0.32-4.65)	
Current	68	11.8	1.34 (0.56-3.21)		19	21.1	1.01 (0.30-3.49)	

similar to other regions of China.³ In Hong Kong, the rate was significantly lower at 5.6%. In Guangzhou, younger women (age <39 years) had a significantly higher infection rate than older women. In Hong Kong, the highest infection rate was in women in the age-groups of 20-29 years and >60 years. The difference in HPV prevalence between the two regions could be due to differences in the living environments and life-styles.

In Hong Kong, the crude HPV infection rate in healthy women was 7.2%, which was lower than that in our previous study.³ The difference could be due to the different HPV detection methods used. The Hybribio HPV genotyping test detects only 21 HPV genotypes, whereas the DNA sequencing method detects more genotypes. In CIN and cancer samples, the HPV prevalence was also significantly lower in our study than in the previous study. These CIN and cancer samples were paraffin-embedded tissues, and the DNA extracted was frequently fragmented. As the primers in the Hybribio HPV genotyping test amplify 450 bp long products, a number of HPV-positive samples and genotypes might have been missed.

HPV16 remained the most common high-risk type detected in both Hong Kong and Guangzhou, and HPV18 was the second most common type in cervical cancer. The frequency of HPV16 increased with increased disease severity. Consistent with our previous finding,³ high prevalence of HPV58 and HPV52 was noted, especially in the normal and precancerous groups.

Having previous cervical cancer screening was an independent risk factor associated with HPV infection in Hong Kong women, but not in Guangzhou women. This might be due to the small sample size. Only one third of women in Guangzhou had a previous Pap smear test, compared with >80% of women in Hong Kong. This might have contributed to the higher incidence of HPV infection in healthy women in Guangzhou. Cervical cancer screening should be promoted to prevent cervical disease.

In Guangzhou, women having ≥3 lifetime sexual partners had increased risk of HPV infection. In Hong Kong, sexual behaviour factors had no such association. A population-based study in Shanxi, China also found no such association.⁴

Integration of HPV16 into the host DNA has been proposed as a potential marker of cervical neoplastic progression.² E2 disruption may occur in any part of the E2 genome, which spans about 1000 bp. To optimise the test sensitivity, we designed five sets of primers to flank the entire E2 sequence for HPV16 or HPV58 genes. Most of the HPV16-positive samples harboured mixed forms of viral integration products. The mixed integrated form of HPV16 was the most prevalent physical state in normal cytology, and the complete integrated form occurred in two thirds of LG CIN cases. Similar findings have also been reported.⁵

In this study, the prevalent physical state of

HPV58 integration was the mixed integrated form. HPV58 commenced early during the course of lesion progression, but this might not be essential for the development of HPV58-related cervical cancer.

Significant differences in HPV16 and HPV58 integration patterns were not observed between Hong Kong and Guangzhou women, although the prevalence for both types was significantly higher in Hong Kong women. Clinical association with abnormal cytology was not observed with integration. This may have been due to the limited number of mixed swap samples and archived paraffin samples analysed. The significant portion of normal cytology samples harbouring the mixed integration form suggested that HPV16 or HPV58 integration occurred early in the cervical transformation process leading to deregulated expression of the E6/7 oncoproteins resulting in major genomic instability in HPV-infected cells. Therefore, detection of viral DNA integration may have prognostic value in the prediction of cervical lesion progression, especially in women without detectable cervical abnormality.

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