

RESEARCH COMMUNICATION

Prevalence and Genotype Distribution of Human Papillomavirus Infections in Women Attending Hospitals in Chaozhou of Guangdong Province

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Abstract

Background: Human papillomavirus (HPV) infection is the main cause of cervical cancer. Limited epidemiologic data of HPV prevalence are available for women attending hospitals in southern China. This study aimed to evaluate the profiles of HPV infection and cytology status in gynecological outpatients in Chaozhou City. **Methods:** A total of 2833 eligible women were enrolled. The HPV GenoArray test was used for HPV detection and genotyping. Nearly one half of the HPV positive women received liquid-based cytology test. Logistic regression analysis was performed to assess the predictable effects of age and genotype for categories of abnormal cytology. **Results:** The prevalence of overall, high-risk, and low-risk HPV infection were 24.5%, 19.5% and 8.4%, respectively. A U-shaped age-specific prevalence curve was observed in overall HPV and high-risk HPV, but not in low-risk HPV, which declined with age increasing. The 6 most common high-risk HPV type in descending order, were types 52, 16, 58, 18, 68, and 33. Age and HPV genotype were both important determinants of abnormal cytology incidence, the older women (>45 years) and those infected with HPV type 16 and/or 18 having the highest risk for abnormal cytology. **Conclusion:** Our findings support the hypothesis that second-generation HPV prophylactic vaccines including HPV-52 and -58 may offer higher protection for women residing in Chaozhou and neighboring cities in Guangdong.

Keywords: Human papillomavirus - prevalence - outpatients - Guangdong, China

Asian Pacific J Cancer Prev, 13, 1519-1524

Introduction

Cervical cancer is the second most common gynecologic malignancy in Chinese women, nearly 200,000 cases being diagnosed annually (Lin et al., 2008; Chen et al., 2011). Certain types of human papillomavirus (HPV) were generally recognized as causative agents in the development of cervical cancer and its precancerous lesions (Bosch et al., 2002; Trottier and Burchell, 2009). More than 100 HPV genotypes had been molecularly characterized, and nearly 40 types were known to infect the genital tract. According to previous report, HPV-16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -69, -73, -59 and -82 were classified as "high-risk types" (Muñoz et al., 2003; Bouvard et al., 2009), and associated with cervical cancer; while HPV-6, -11 -42, -43, -44 were classified as "low-risk types" (Middleton et al., 2003; Trottier and Burchell, 2009), which were rarely detected in high-grade cervical lesions but associated with anogenital warts.

The relationship between HPV infection and cervical

cancer had given great impetus to the development of prophylactic vaccines against the most common HR-HPV types. Since HPV16 and 18 accounted for approximately 70% of cervical cancer cases worldwide (Muñoz et al., 2003), two licensed HPV vaccines; Gardasil (HPV6/11/16/18) (Villa et al., 2005) and Cervarix (HPV16/18) (Harper et al., 2004) were developed against these two major cancer-causing HPV types (Yoshikawa, 2009). Meanwhile, most interest had been raised to determine age-related HPV infection and to identify the major genotypes in different countries. Based on the meta-analysis reported worldwide, HPV type 16 and 18 were the most common HPV types in Europe and Africa (de Sanjosé et al., 2007; Bruni et al., 2010), following was types 45, 31 and 33, whereas HPV 52 and HPV 58 presented a more frequent prevalence in Asia (Bao et al., 2008; Sun et al., 2010; Chen et al., 2012).

In southern China, a few epidemiologic researches had been performed to evaluate the HPV prevalence and genotype distribution in asymptomatic female

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population (Lin et al. 2008; Wu et al., 2010; Yip et al., 2010; Chen et al., 2012), but little data was available from outpatients (Lin et al., 2006; Xue et al., 2009). Due to the accessible anatomic site for sample collection and the well-established sample collecting method for cervix, we investigated the cervical HPV infection in women attending hospitals, to understand the age-specific and genotype-specific prevalence of HPV in Chaozhou City in recent 2 years, and to collect timely and sufficient information to support future vaccination program.

Materials and Methods

Study population

Chaozhou City located in easternmost Guangdong province of the People's Republic of China. From January 2010 to December 2011, the study was simultaneously carried out in 3 local hospitals (Chaozhou Central Hospital, Chaozhou People's Hospital, Chaozhou Gynecological and Pediatric Hospital). A woman was eligible to be study subject if she: (a) was a gynecological outpatient and with genital tract disease related symptom (commonly was cervicitis and/or vulvar discomfort); (b) was not presently pregnant; (c) had not undergone a total uterus or cervix resection; and (d) was willing to undergo HPV testing and consent to participate in the present study. The study was carried out with the approval of the ethical committee of 3 above-mentioned hospitals, and patients consent was obtained for the collection of cervical exfoliated cells.

Cervical specimen collection and HPA DNA extraction

Eligible outpatients underwent a gynecological examination performed by gynaecological practitioners in 3 above-mentioned hospitals. During the examination, samples of exfoliated cervical cells were collected using plastic cervical swabs. The sampler was inserted 1-1.5 cm into the endocervical canal and rotated 4-5 full turns in counter-clockwise direction. The tip containing cellular material was then placed into transport medium tube and stored at 4 °C immediately. All swabs and store bottle with specimen transport medium were from HybriBio Biotechnology Limited Corp., Chaozhou, China. The brush and supernatant were removed after the cells were centrifugated for 5 mins with relative centrifugal force of 960 g. DNA was extracted from the sediments with alkaline lysis method Kits (HybriBio Biotechnology Limited Corp.) (Klitschar and Neuhuber, 2000; Chen et al., 2012).

HPV GenoArray test

HPV GenoArray test was used for HPV detection and genotyping. Genotyping was done by DNA amplification, flow-through hybridization and gene chip by HybriMax (HybriBio Biotechnology Limited Corp., Chaozhou, China). Test was performed according to the manufacture's instructions. Detailed protocols for this assay had been described previously (Lin et al., 2008). The gene chip contained type-specific oligonucleotides immobilised on a nylon membrane. The chip could identify 14 HR-HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66 and 68) and 7 LR-HPV types (6, 11, 42, 43, 44, 53 and

81) (Dunne et al., 2007). The final results were detected by colorimetric change on the chip under direct visualization (Lin et al., 2008).

Liquid-based cytology test (LCT) and pathological diagnosis

HPV positive outpatients were advised to receive LCT, and 113 HPV negative outpatients received LCT simultaneously. The outpatients were called back and exfoliated cervical cells were collected, mixed with 3 mL specimen stored liquid (HybriBio Biotechnology Limited Corp., Chaozhou, China) and stored in room temperature. The specimens were sent to Chaozhou Central Hospital for LCT analysis. The results were evaluated using the Bethesda system (Solomon et al., 2002). The evaluation system included: (i) negative (A0), (ii) atypical squamous cells (ASC), (iii) low-grade squamous intraepithelial lesion (LSIL), (iv) high-grade squamous intraepithelial lesion (HSIL), and (v) squamous cell carcinoma (SCC).

Statistical analysis

For age-specific HPV prevalence assessment, the eligible females were divided into 6 age groups (<26, 26-30, 31-35, 36-40, 41-45 and >45 years) with five-year interval, the actual age of each study subject was counted with the formula: (interview date - birthday) / 365.25 by Microsoft Excel software. The prevalence of overall and type-specific HPV infections as well as their corresponding 95% confidence intervals (95% CI) were estimated by binomial distribution analysis. Chi-squared tests were used to assess the statistical significance of any differences in prevalence. The binary and multinomial logistic regression model were performed to identify variables that associated with cervical lesions, and the odd ratios (OR) and their 95% CI were calculated. All data were analyzed using SPSS software version 16. P values were two-sided, and statistical significance was accepted if the P value was 0.05 or less.

Results

From January 2010 to December 2011, a total of 2947 outpatients received HPV testing in 3 above-mentioned hospitals. 2833 eligible females were enrolled into this study, 114 cases were excluded, since they did not consent to participate in the study or did not meet the inclusion criteria.

Among 2833 eligible women (age ranged from 16.3 to 71.3 years, mean age 35.2±9.0 years), genotyping test revealed that 694 cases were positive for HPV infection, and the overall prevalence of HPV was 24.5% (694/2833, 95% CI: 22.9-26.1%). 551 (19.5%, 95% CI: 18.0-20.9%) cases were infected with at least one HR-HPV type, and 237 (8.4%, 95% CI: 7.1-9.1%) cases were infected with one or more LR-HPV type. Nearly three quarters (517/694, 74.5%) of HPV positive women were infected with single HPV type, the others (177/694, 25.5%) presented multiple HPV types infection.

Age-specific prevalence of HPV infection

A U-shaped age-specific prevalence curve was observed in Overall HPV prevalence (Figure 1A).

Table 1. Age-specific Prevalence of HPV Infection

Age (years)	Total case		Overall HPV		HR-HPV		LR-HPV			
	No.	Positive case No.	Prevalence (%)	Prevalence (95% CI) %	Positive case No.	Prevalence (%)	Prevalence (95% CI) %	Positive case No.		
<26	459	175	38.13	(33.76-42.63) ^a	125	27.23	(23.29-31.42) ^c	93	20.3	(16.8-24.1) ^d
26-30	484	91	18.80	(15.49-22.44)	62	12.81	(10.03-15.98)	43	8.88	(6.57-11.6)
31-35	520	89	17.12	(14.04-20.51)	66	12.69	(10.02-15.73)	30	5.77	(3.98-7.99)
36-40	572	132	23.08	(19.75-26.65)	111	19.41	(16.31-22.78)	25	4.37	(2.90-6.25)
41-45	431	96	22.27	(18.52-26.36)	86	19.95	(16.36-23.90)	19	4.41	(2.73-6.62)
>45	367	111	30.25	(25.69-35.07) ^b	101	27.52	(23.12-32.24) ^c	17	4.63	(2.79-7.10)
Total	2833	694	24.50	(22.94-26.10)	551	19.45	(18.02-20.93)	237	8.37	(7.05-9.05)

^aThe prevalence was significantly higher than that of other age groups ($P = 0.018$ for age >45 group, $P < 0.01$ for all middle-aged groups); ^bThe prevalence was significantly higher than that of middle-aged groups ($P < 0.05$ for all); ^cBoth prevalence was significantly higher than that of middle-aged groups ($P < 0.05$ for all), but no statistical difference was observed between themselves ($P = 0.927$); ^dThe prevalence was significantly higher than that of other age groups ($P < 0.01$ for all); HR-HPV, high-risk human papillomavirus; LR-HPV, low-risk human papillomavirus; 95% CI, 95% confident intervals

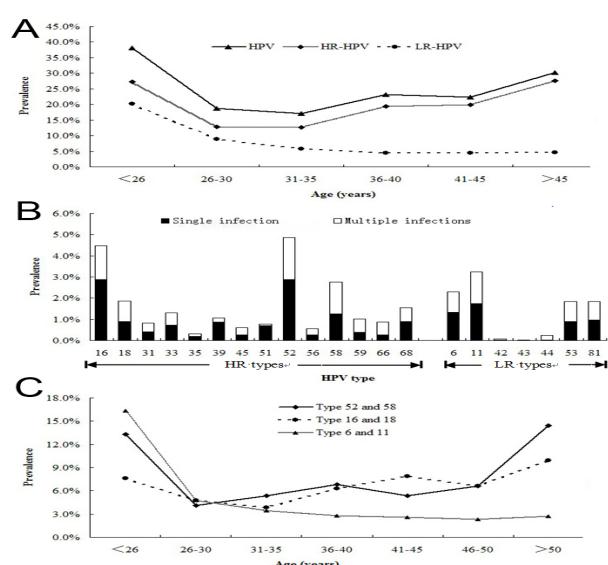


Figure 1. Age and Genotype-specific Prevalence of HPV. A: age-specific prevalence of overall, high-risk and low-risk HPV; B: genotype-specific prevalence of HPV; C: age-specific prevalence of type 16 plus 18, type 52 plus 58, and type 6 plus 11

Outpatients below the age of 26 years had the highest HPV prevalence (38.1%), which was significantly higher than that of other age groups (Table 1). A less pronounced second peak of HPV infection was found in women older than 45 years of age (30.2%), which was significantly higher than that of middle aged groups (age ranged from 26 to 45 years), but lower than the first peak prevalence ($P=0.018$) (Table 1). The HPV prevalence of middle-aged groups ranged from 17.1 % to 23.1 %, lower than the overall prevalence (24.5%), and there was not significant difference among 4 middle-aged groups ($P=0.431$).

The patterns of age-specific prevalence of HR-HPV were similar to that of overall HPV prevalence (Figure 1A). 2 peak prevalence was observed at the youngest (27.2%) and the oldest (27.5%) age group respectively, and both of them were all significantly higher than that of middle-aged groups ($P < 0.05$ for all) (Table 1).

The age-specific prevalence of LR-HPV was greatly different from that of HR-HPV. The peak prevalence was only found in the youngest age group (20.3%), and it was statistically higher than that of other groups ($P < 0.001$ for all). The prevalence sharply declined in the women older

Table 2. Distribution for HPV Genotypes

Genotypes	Total cases (No.)	Mean age (years)	Prevalence (%)	95% CI (%)
HR types	551	36.4 ± 10.0	19.45	18.02 - 20.93
52	142	34.0 ± 11.1	5.01	4.25 - 5.86
16	131	36.1 ± 10.4	4.62	3.89 - 5.44
58	82	34.4 ± 10.7	2.89	2.32 - 3.55
18	57	35.2 ± 10.5	2.01	1.54 - 2.57
68	48	32.9 ± 9.4	1.69	1.26 - 2.21
33	41	36.4 ± 10.6	1.45	1.05 - 1.93
39	34	33.2 ± 10.1	1.2	0.84 - 1.65
59	33	30.2 ± 11.0	1.16	0.81 - 1.61
66	29	28.8 ± 11.3	1.02	0.70 - 1.44
31	27	33.3 ± 9.8	0.95	0.64 - 1.36
51	26	34.0 ± 8.9	0.92	0.61 - 1.31
45	21	31.2 ± 10.8	0.74	0.47 - 1.10
56	20	36.2 ± 9.6	0.71	0.44 - 1.06
35	13	39.2 ± 3.8	0.46	0.25 - 0.75
LR types	237	29.9 ± 9.9	8.37	7.38 - 9.42
11	92	29.1 ± 9.1	3.25	2.64 - 3.94
6	65	28.1 ± 10.6	2.29	1.79 - 2.89
53	52	31.3 ± 9.2	1.84	1.38 - 2.37
81	52	31.3 ± 10.5	1.84	1.38 - 2.37
44	7	32.4 ± 10.0	0.25	0.11 - 0.48
42	2	24.5 ± 2.0	0.07	0 - 0.22
43	1	28.1	0.04	—
Total	694	35.21 ± 9.0	24.5	22.94 - 26.10

than 25 years of age (Table 1, Figure 1A).

Genotype distribution

The 6 most common HR-HPV types observed in this study in descending order, were types 52 (5.0%), 16 (4.6%), 58 (2.9%), 18 (2.0%), 68 (1.7%), and 33 (1.5%) (Table 2, Figure 1B). The combined prevalence of types 52 and 58 accounted for 7.6% of total cases, and presented a bimodal age distribution. The prevalence of type 16 plus 18 presented 6.6% of total cases, but no obvious peak prevalence could be found on the age-specific prevalence curve (Figure 1C).

The most common LR-HPV types were types 11 (3.3%) and 6 (2.3%) (Table 3, Figure 1B). More than three quarters (152/198, 76.8%) of LR-HPV positive cases were infected with types 6 and/or 11, and there was a peak incidence at the youngest group (Figure 1C).

172 study subjects presented multiple HPV types infection, 122/172 (70.9%) cases were double infections,

Table 3. Effects of Age and Genotype on the Risk of Abnormal Cytology in HPV Positive Female

Variables	Cases (No.)	Mean age (years)	Cytological abnormalities			Total No. of cytological abnormalities (%)	OR (95% CI)
			ASC (No.)	LSIL (No.)	HSIL a (No.)		
Age (years) (No.=319), P < 0.01							
<26	59	22.1 ± 2.3	10	2	0	12 (20.3)	Referent
26-30	41	28.0 ± 1.4	8	1	0	9 (22.0)	1.10 (0.42-2.92)
31-35	44	32.8 ± 1.4	8	1	1	10 (22.7)	1.15 (0.45-2.97)
36-40	76	38.3 ± 1.3	12	7	4	23 (30.3)	1.70 (0.36-3.79)
41-45	53	42.5 ± 1.3	14	5	2	20 (37.7)	2.37 (1.02-5.51)
>45	46	51.6 ± 5.5	14	6	4	25 (54.3)	4.66 (1.97-11.01)
Single HR-type infection (No.=213), P=0.41							
52	58	37.8 ± 10.2	12	4	0	16 (27.6)	Referent
16	55	37.1 ± 9.7	11	7	6	24 (43.6)	2.03 (0.93-4.46)
58	20	35.6 ± 6.4	4	1	1	6 (30.0)	1.13 (0.37-3.44)
18	11	40.2 ± 9.3	3	1	1	5 (45.5)	2.19 (0.59-8.18)
33	10	35.9 ± 9.0	2	1	0	3 (30.0)	1.23 (0.26-4.89)
39	17	34.2 ± 11.5	3	2	0	5 (29.4)	1.09 (0.33-3.60)
Other HR types	42	38.1 ± 9.7	9	1	0	10 (23.8)	0.82 (0.33-2.05)
Type 52 and/or 58 infection vs. type 16 and/or 18 infectionb (No.=202), P < 0.01							
52 and/or 58	112	36.4 ± 10.4	21	6	2	29 (25.9)	Referent
16 and/or 18	90	37.2 ± 9.8	21	10	8	39 (43.3)	2.19 (1.21-3.96)
Single vs. multiple infection (No.=319), P=0.46							
Single infection	213	36.7 ± 9.6	44	17	8	69 (32.4)	Referent
Multiple infection	106	33.6 ± 10.2	22	5	3	30 (28.3)	0.82 (0.49-1.37)
Total (No.=432), P < 0.001							
Control	113	35.9 ± 7.6	2	0	0	2 (1.8)	Referent
HPV positive	319	36.2 ± 9.8	66	22	11	99 (32.5)	24.98 (6.05-103.15)

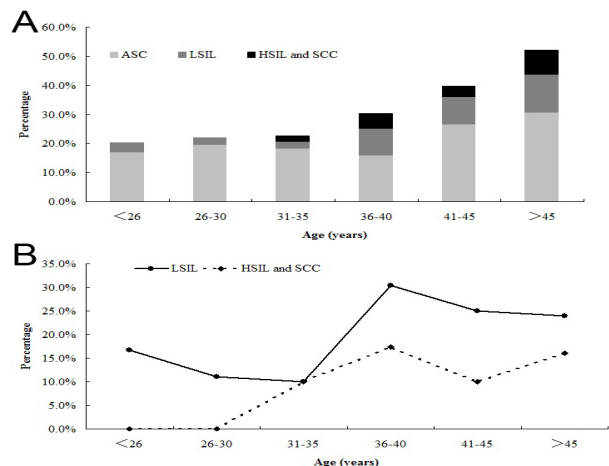


Figure 2. Age-specific Prevalence of Cytological Abnormalities in HPV Positive Females. A: age-specific prevalence of cytological abnormalities in HPV positive females; B: age-specific trends for the percentage of LSIL and HSIL/SCC in cytological abnormalities

31/172 (18.0%) cases were triple infections, and the others (19/172, 11.0%) were quadruple infections or more. At most, 2 cases were infected with 6 different subtypes. Of these 172 multiple infections females, 67 (39.0%) presented multiple HR-HPV infections, and 11 (6.4%) exhibited multiple LR-HPV infections, the others (94/172, 54.7%) were combined infection of HR-HPV and LR-HPV types.

Cytology test

In accordance with the suggestion of gynaecological practitioners, a total of 319 HPV positive women (319/694, 46.0%) and 113 HPV negative women (control study) received LCT. For control group, only 2 ASC cases (2/113, 1.8%) were diagnosed, the others were all

negative for cytology test. However, the percentage of abnormal cytology in the HPV positive population was 31.0% (99/319), which was extremely higher than that of control group statistically (P < 0.001).

The percentage of cytological abnormalities increased with the age increasing in HPV positive females (Figure 2A). The most common abnormality was ASC in whole cohort, while the percentage of LSIL, HSIL and SCC increased in the women older than 35 years (Table 3, Figure 2B).

The HPV genotype specific prevalence of cytological abnormalities was summarized in Table 3. For single infection, the women who infected with HPV type 16 or 18 had higher risk for cytological abnormalities compared to type 52 carriers (OR = 2.03, 95% CI: 0.93-4.46 for type 16; OR = 2.19, 95% CI: 0.59-8.18 for type 18), but there was not statistical difference (P = 0.067 for type 16; P = 0.096 for type 18). However, the significant difference was found between the women who infected with type 16 and/or 18 compared to those who infected with 52 and/or 58 (the cases that heterozygous with 52 or 58 and 16 or 18 infection were excluded). The former had 2 fold risks with abnormal cytology than the later (OR = 2.19, 95% CI: 1.21-3.96, P = 0.009). In addition, two SCC cases found in this study were all HPV type 16 single infection.

Discussion

In this study, we investigated the profiles of HPV infection and cytology status in Chaozhou City. The sample population were all attending hospitals women, and most of them (2006/2833, 70.8%) were clinical diagnosed cervicitis. Compared with similar studies in southeast China, the overall HPV prevalence (24.5%) measured here was similar to that reported in Shanghai

(30.2%) (Xue et al., 2009) and Kaohsiung (19.3%) (Lin et al., 2006).

The age of whole study population ranged from 16.3 to 71.3 years, but only 3.6% (101/2833) women were aged 20 or younger, and 3.9% (111/2833) women aged 51 or older, therefore, these 2 cohorts were attribute to the youngest (<26) and oldest (>45) age group, respectively. After all data linear arrayed by the age, a paralleled bimodal curves were observed in the prevalence of overall HPV and HR-HPV, but not in LR-HPV, which declined with age increasing (Figure 1A). The younger women had the highest HR-HPV and LR-HPV prevalence. As HPV was often acquired soon after sexual initiation, the high prevalence was suggested to be a reflection of the changes in the sexual behaviours (Liu et al., 2011; Yang et al., 2003). Compared with vaccines Cervarix, which was restricted to the HPV types 16 and 18, the Gardasil would offer higher protection for younger women, because it could provide additional protection for type 6 and 11, the 2 most common LR types and with highest prevalence in youngsters. The second peak prevalence of HR-HPV was observed in women aged 45 or older, which was hypothesized the results of reactivation of latent infections due to impaired immune response (de Sanjosé et al., 2007).

The most common HR-HPV subtypes in general women population worldwide were HPV-16, -18, -31, -58, -52, -51 and -33 (de Sanjosé et al., 2007), while in some Asian countries, the HPV-52 and HPV-58 infections were as common as HPV16 infection (Inoue et al., 2006; Liu et al., 2010; Ye et al., 2010; Chen et al., 2012). Similar prevalence pattern also exhibited in our sample population, HPV-52 was the most commonly identified HR type, following were types 16, 58, 18, 68 and 33. Furthermore, we found that the prevalence of type 52 plus 58 (7.6%) was 1 percent higher than that of type 16 plus 18 (6.6%). Interestingly, the age-related bimodal distribution was found in type 52 plus 58, but no obvious peak was observed in type 16 plus 18. For the current available prophylactic vaccines, limited cross-protection could be offered between HPV types (Herrero, 2009), heterogeneity in HPV type-specific distribution from different populations should be taken into account. Therefore, the second-generation vaccines should target to specific regions (Clifford et al., 2005). Our findings supported the hypothesis that the second-generation HPV prophylactic vaccines including HPV-52 and -58 may offer higher protection for women residing in Chaozhou and cities in the neighborhood (Chen et al., 2012).

In order to explore the predictable effects of age and genotype for categories of abnormal cytology, nearly one half (46%) HPV positive women received liquid-based cytology test, and 113 HPV negative women were set as control study. As expected, the HPV positive females had almost 25 fold of risk for abnormal cytology than the control group. Logistic regression model revealed that age and HPV genotype were all important determinants of abnormal cytology incidence. The older women and those who infected with HPV type 16 and/or 18 may belong to a group of women with high risk for abnormal cytology. The incidence peak observed in older women may be partially explained by viral persistence and immunologic disorder

caused by hormone fluctuations at menopausal transition (Hildesheim and Wang, 2002; Chen et al., 2012), and the incidence peak found in HPV type 16 and/or 18 infection was coincident with the fact that HPV16 and 18 were the most common types found in invasive cervical cancer (ICC) worldwide (de Sanjosé et al., 2007).

In conclusion, the high prevalence of HPV52 and 58 observed in our study, supported the hypothesis that the second-generation HPV prophylactic vaccines including HPV-52 and -58 may offer higher protection for women residing in Chaozhou and neighboring cities. Young age was the most prominent independent risk factor not only for HR-HPV but also for LR-HPV infection, the vaccine Gardasil, rather than Cervarix, was more competent for this cohort. The old women had peak prevalence of HPV infection and abnormal cytology, the constructive screening program including HR-HPV detection, cytology evaluation and even colposcopy, should be considered regular screening and should be prolonged because they had higher risk for the development of cervical cancer.

Acknowledgements

This work was supported by Medical Research Funds of Guangdong Province (A2011760, B2008179). Reagent discounts and reagent gifts was from HybriBio Biotechnology Limited Corp.. The funders had no role in study design and data analysis, decision to publish, or reparation of the manuscript. We sincerely thank all the staff and participants who took part in this study. We offer special recognition for the excellent work of the study staff in sample collection. We declare that we have no any potential conflicts of interest with other people or organizations.

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