

## RESEARCH ARTICLE

# Human Papillomavirus (HPV) Infection in Women Participating in Cervical Cancer Screening from 2006 to 2010 in Shenzhen City, South China

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### Abstract

**Purpose:** Human papillomavirus (HPV) infection plays an important role in the development of cervical cancer, but the prevalence of HPV infection in women of Shenzhen city remains unclear. The present study was performed to describe the change of cervical HPV infection in females who participated in voluntary cervical cancer screening from 2006 to 2010 in Shenzhen city, China. **Methods:** A total of 4,413 women were recruited. HPV infections were genotyped by polymerase chain reaction (PCR) and reversed dot blot hybridization in Shenzhen Maternity and Child Health Hospital. **Results:** The prevalence of HPV infection was 13.8%. The five most commonly found HPV types were HPV16 (3.47%), HPV58 (1.68%), HPV33 (1.38%), HPV43 (1.36%) and HPV18 (1.27%). The secular trends of major HPV type-specific were diverse. Among of them, the prevalence of HPV18 increased sharply while others increased slowly or even decreased in the period. The change of total HPV, single HPV and multiple HPV infection were similar during the five years. **Conclusions:** Our findings suggested that HPV infection is common with HPV16 and HPV 58 as the primary subtypes in women in Shenzhen city. The prevalence of HPV 18 infection is increasing faster than any others, which will lead it to be one of the main subtypes in this city in the future.

**Keywords:** Cervical cancer - human papilloma virus - subtype - epidemiology - China

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### Introduction

Invasive cervical cancer (ICC) is the third most common cancer among women worldwide (Bernard et al., 2013). In the middle of the 1970s, the hypothesis that cervical cancer may arise from virus infection was established, and in the 1990s, the causal relationship between genital human papilloma virus (HPV) infection and cervical cancer was confirmed (Hausen et al., 1996; Bosch et al., 2002). The most significant etiological factor, HPV in the development of both invasive cervical cancer (ICC) and its precursor lesions (cervical intraepithelial neoplasia, CIN) has been well established (Walboomers et al., 1999; Bosch et al., 2003). It is now widely recognized that HPV infection is a necessary cause for over 99% of cervical cancer cases (Hausen et al., 1999; Clifford et al., 2006), and nearly all invasive cervical cancers are indeed positive in HPV DNA test. HPV is a large of group of epitheliotropic viruses of more than 200 different subtypes. Among them, only 40 HPV subtypes could infect the human being. According to its potential to induce carcinogenesis, HPV types have been classified as low-risk oncogenicity (LR-HPV) and high-risk

oncogenicity (HR-HPV) (Munoz et al., 2003). Previous studies showed that HR-HPV plays a crucial role in the etiology of anogenital cancer, especially cervical cancer (Cavalcanti et al., 1996). Many studies had showed that 13 HR-HPV subtypes were significant connected with cervical cancer, namely HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68 (Clifford et al., 2003; Hwang et al., 2003). Among the high-risk types, infection with HPV16 and 18 are associated with significantly higher risk of disease progression, and consequently these two types together cause approximately 70% of invasive cervical cancer worldwide (Carter et al., 2000; Smith et al., 2007). The distribution of HPV genotypes in invasive cervical cancer is crucial to guide the introduction of prophylactic vaccines as well. Two prophylactic HPV vaccines are currently available and protect against two carcinogenic HR-HPV types (HPV16 and HPV18). One is a Merck's quadrivalent vaccine preventing infection from 4 HPV types (HPV 6, 11, 16, and 18) (Gardasil), and was approved by FDA in 2006. The second is a bivalent vaccine preventing infection from 2 high-risk oncogenic HPV types (HPV16 and 18) (Cervarix). The two prophylactic HPV vaccines have been licensed in the

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**Table 1. The Characteristics of Subjects in the Cervical Cancer Screening in Shenzhen City, 2006-2010**

	Number of subjects with HPV analysis	Number of cases with HPV positive	Crude HPV prevalence % (95% CI)
Age (years)			
≤25	387	84	21.71 (17.70 - 26.15)
26-35	1961	269	13.72 (12.22 - 15.32)
36-45	1525	180	11.80 (10.23 - 13.53)
≥46	540	76	14.07 (11.25 - 17.30)
Date of diagnosis			
2006	1278	105	8.22 (6.77 - 9.86)
2007	519	68	13.10 (10.32 - 16.31)
2008	687	127	18.49 (15.65 - 21.59)
2009	1050	159	15.14 (13.03 - 17.46)
2010	879	150	17.06 (14.63 - 19.72)

95% CI, 95% Confidence Interval

United States, Europe, Australia, Latin America (Garland et al., 2008). HPV prophylactic vaccines are successfully developed and applied to the primary prevention of cervical cancer. However, HPV genotypes show the different distribution in different regions and populations. According to a Meta-analysis, HPV16 and 18 were the two most common genotypes among Asian women with invasive cervical cancer (ICC). Meanwhile, HPV52 and HPV58 were more common, with a suggestion of a higher proportion of HPV52 and 58 among ICC cases from eastern and southeastern Asia when compared to other geographical regions (Smith et al., 2007; Bao et al., 2008). This finding suggests that HPV bivalent vaccine or HPV quadrivalent vaccine exist some limitations. HPV genotypes diversity and its multiple infections may increase the complexity of cervical cancer prevention.

Although previous studies have confirmed that cervical cancer is associated with genital human papilloma virus (HPV) infections, but important regional variations have been found. Meanwhile there is no large sample data on the prevalence of HPV infection and HPV genotypes from south of China. Therefore, the purpose of this population-based HPV type prevalence study was to explore the distribution of cervical infection HPV genotypes, and then analyze the epidemiological diversity of HPV in Shenzhen city, south of China.

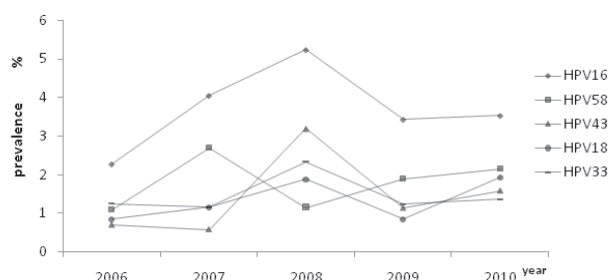
## Materials and Methods

### Study population

All participants are women who voluntarily participated cervical cancer screening from 2006 to 2010 in Shenzhen Maternity and Child Healthcare Hospital. A total of 4,413 females were enrolled by screening records. The inclusion criteria: woman with age of 15 years and more. Exclusion criteria: history of cervical surgery, hysterectomy, vaginal diseases surgery or pelvic radiation therapy history. The study protocol was approved by the Shenzhen Maternity and Child Healthcare Hospital Medical Ethics Committee, and informed consent was obtained from all participants.

### Laboratory measurement

Cervix cast-off cells were collected by a cervical

**Figure 1. The Changes of HPV Type-specific Prevalence from 2006 to 2010 in Shenzhen City**

cytobrush, and soaked in 2 ml Preserv Cyt solution (U.S. Cyt Company) together with the cytobrush. The biological samples were stored at 4°C, and then sent to the laboratory for detection. PCR and reverse dot blot hybridization oligonucleotide probes (reverse dot blot, RDB) were applied to determine subtypes of HPV, including with cervical cancer and cervical intraepithelial neoplasia (CIN) is closely related to 13 kinds of high-risk subtypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) and screened according to the literature of the other 10 common subtypes (6, 11, 42, 43, 44, 53, 66, 73, 83, and MM4), HPV genotyping was performed using HPV genotyping test kit (Yaneng) BIOscience (Shenzhen Co., Ltd. China) according to the manufacturer's instructions.

### Statistical analysis

All analyses were performed using the STATA software (version 11.0, STATA Corp, College Station, TX). The prevalence of total HPV and HPV subtypes were expressed as percentage. Chi-square test was used to compare the prevalence of HPV infection in different age groups and years. All reported P-values were two-sided, and P-values of less than 0.05 were considered to be statistically significant.

## Results

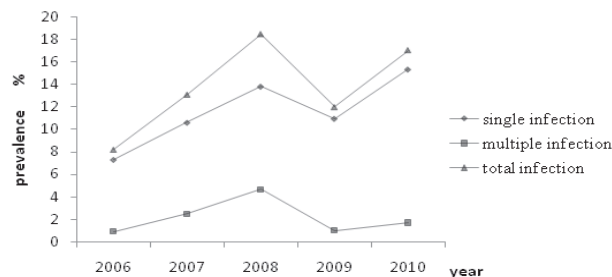
A total of 4413 cases were recruited in this study, of which 8.77% women being age group ≤ 25-year old, 44.44% women belonging to the age group of 26-35 years old, 34.56% women being 36-45 years old and 12.23% women being more than 46 years old. The prevalence of infection with any HPV type was 13.80% (609/4413). Average age of the positive persons was 40.34 years old. Characteristics of the study populations are shown in Table 1. For age groups, HPV prevalence ranged from 21.71% in the youngest age group to 14.07% in the oldest age group. It was significantly higher in age group ≤ 25-year old than that in other groups. And the other three age groups exhibited no significantly different in HPV prevalence. During the past five years, the prevalence of HPV in 2006 was significantly lower than that in other years. It was not significantly different from 2007 to 2010.

Except for HPV44, the rest 22 genotypes were detected in the study. The five most commonly found HPV types were HPV16 (3.47%), HPV58 (1.68%), HPV33 (1.38%), HPV43 (1.36%) and HPV18 (1.27%). The secular trend of prevalence of major HPV type-specific was showed in Figure 1. The prevalence of HPV16 had been significantly

**Table 2. The Distributions of Major HPV Subtypes in Subjects in the Cervical Cancer Screening in Shenzhen City, 2006-2010**

Infection type	HPV type-specific positive ICC cases n	HPV type-specific relative contribution %
Single		
HPV16	107	17.57 (14.63 - 20.83)
HPV58	58	9.52 (7.31 - 12.14)
HPV33	41	6.73 (4.87 - 9.02)
HPV43	40	6.57 (4.73 - 8.84)
HPV52	37	6.08 (4.31 - 8.28)
Multiple types		
HPV16&OT	46	7.55 (5.58 - 9.95)
HPV18&OT	23	3.78 (2.41 - 5.61)
HPV33&OT	22	3.61 (2.28 - 5.42)
HPV68&OT	20	3.28 (2.02 - 5.03)
HPV43&OT	20	3.28 (2.02 - 5.03)
HPV58&OT	17	2.79 (1.63 - 4.43)

OT, one or more other HPV types

**Figure 2. The Changes of HPV Total and Subtype Infections Prevalence from 2006 to 2010 in Shenzhen City**

higher than that of others from 2006 to 2010. And during the past five years, the prevalence of HPV16, HPV33, HPV43 and HPV18 exhibited a peak in 2008 while HPV58 in 2007. The bottom of HPV16, HPV33, HPV43 and HPV18 were in 2009 while HPV58 in 2008. After 2009, prevalence of all major HPV type-specific increased slowly.

The prevalence change of single and multiple HPV infection from 2006 to 2010 was showed in Figure 2. The line chart showed that the prevalence of single HPV infection was significantly higher than that of multiple HPV infection. All of total HPV infection, single HPV infection and multiple HPV infection exhibited a peak in 2008 and a bottom in 2009, which indicated that the change tendencies of total HPV infection, single HPV infection and multiple HPV infection were consistent during the past five years.

Table 2 shows the type-specific HPV distribution in subjects with the invasive cervical cancers screening. Most common types (among single HPV-positive cases) were HPV16 (17.57%), HPV58 (9.52%), HPV33 (6.73%), HPV43 (6.57%) and HPV52 (6.08%). However, there were some differences among multiple HPV infection. As one of the killer in multiple infection, HPV16 was the most common type, followed by HPV18, HPV58, HPV33, HPV56 and HPV43.

The prevalence change of single HPV infection and multiple HPV infection from 2006 to 2010 in different age group in Shenzhen city was showed in Figure 3A and

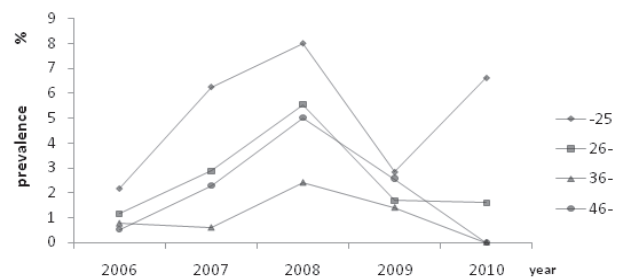
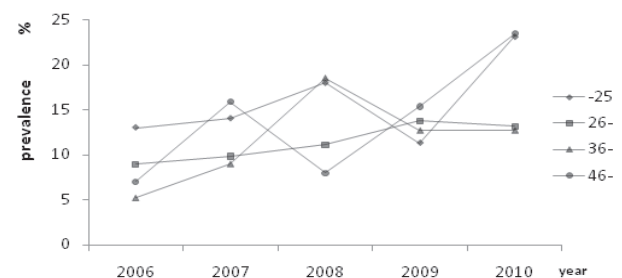
**Figure 3. The Changes of Single HPV Infection (a) and Multiple HPV Infections (b) Prevalence from 2006 to 2010 in Different Age Groups in Shenzhen City****Figure 4. The Changes of Single HPV Infection (a) and Multiple HPV Infections (b) Prevalence from 2006 to 2010 in Different Age Groups in Shenzhen City**

Figure 3B. For single HPV infection, the HPV prevalence exhibited a peak in 2008 and a bottom in 2009 for age groups  $\leq 25$  years old and 36-45 years old, a peak in 2007 and a bottom in 2008 for age groups  $\geq 46$  years old. However, the HPV prevalence in age group 26-35 years old had been increasing steadily. For multiple HPV infection, the HPV prevalence exhibited a peak in 2008 and a bottom in 2009 for age groups  $\leq 25$  years old. The HPV prevalence in age groups 26-35, 36-45 and  $\geq 46$  years old exhibited a peak in 2008, then decreased shapely from 2008 to 2010. This indicated multiple HPV infection in age groups 26-35, 36-45 and  $\geq 46$  years old was under control.

## Discussion

Our findings showed a relatively high prevalence of HPV (13.80%) among females in Shenzhen city, and similar ratios were found in Taipei and five areas of mainland China, but higher than that reported in North India, and lower in Italy (Datta et al., 2010; Wang et al., 2010; Guido et al., 2013; Wu et al., 2013). The reasons could be explained by the different detection methodologies, and the ethical, racial, or geographic diversity. The further analysis showed that the prevalence of HPV from 2006 (8.22%) was significantly lower than that in other years, which suggested that HPV infection status may be different at different times in the same area. The high prevalence of HPV and its rising trend should attract more attentions in society.

The prevalence of HPV infection in different age groups was very diverse (Wang et al., 2010). This study showed that the prevalence of HPV was higher in the younger age groups than the older ones, and the highest HPV prevalence was found in age group  $\leq 25$ -years old, and increasing ages were significantly associated with the

decreasing prevalence of HPV infection from 26 to 45 years old, which was consistent with the published results (Cibas et al., 2007). Previous study reported the decrease of HPV prevalence in older women may be related to a biological effect, such as increased immunity to HPV with aging (Figuerola et al., 1995). However, HPV prevalence in age group  $\geq 46$  years old was a little higher than age group 26-35 and 36-45 years old, which may due to selection bias in old population. In addition, a recent study reported that HPV infection in younger women was mostly transient infection, and only persistent infection was a risk factor for cervical cancer (Bosch et al., 1995). Influencing factors of higher HPV prevalence and the infection belonging to transient or persistent infection in young women needs to be deeply studied in the future.

HPV subtypes distribution characteristic is one of focal issues in cervical cancer prevention and treatment study, which relates to the screening of cervical cancer and precancerous lesions, and will exert a direct impact on the development and application of cervical cancer preventive vaccine. The present study found that high-risk HPV was common in Shenzhen females and the five most common types were HPV16, 58, 33, 43 and 18. Among them, HPV16 was the first common subtype, which was consistent with the relevant reports from other groups. HPV58, HPV33 and HPV43 sorted priority to HPV18, which were more common in Asia, but relatively rare in other areas of the world (Wright et al., 2000; Wu et al., 2006). Among single HPV subtype infections, HPV16, HPV58, HPV33, HPV43 and HPV52 were the common types. HPV52 were the predominant in the present study, which was consistent with the domestic reports (Bao et al., 2007; Liu et al., 2007; Wu et al., 2013). HPV52 undoubtedly has great significance for the new generation of vaccine development, application and cervical cancer prevention and control. Therefore, the application of the preventive vaccine mainly against both HPV16 and HPV18 in Chinese populations still need to be further investigated. Multiple-type infections are not uncommon in women in Shenzhen city. However, the proportion of multiple-types was inconsistent with previous reports, which may be related to regional and ethnic differences (Nielsen et al., 2008; Mejlhede et al., 2009; Chaturvedi et al., 2011). In our study, multiple-type infections accounted for 14.78%. Among the multiple-types, HPV16, 18, 33 were most common and accounted for 7.55%, 3.78% and 3.61% of total infected cases, respectively. The above signs further showed that HPV gene distribution characteristics had difference in different infection type and regions as well (Einstein et al., 2002; Munoz et al., 2003; Hibbitts et al., 2008; Oh et al., 2009). The development and effective application of preventive vaccine undoubtedly will be more complex due to the multiple-types infection. Whether the multiple infections increased the risk of cervical cancer occurring is still not clear, which needs to be further deeply investigated. Furthermore, we also showed that the prevalence of multiple-types infection is higher in young group than the older group. In addition, the prevalence of HPV infection was higher in younger age groups. This indicated that cervical cancer prevention should be focused on younger female populations.

Although the present study has a relative large sample size and described HPV and HPV subtypes prevalence in Shenzhen females, a limitation should be noted. Namely, a selection bias might exist for study subjects only came from women who voluntarily participated cervical cancer screening in a maternity and child healthcare hospital. Considering the subjects in the study came from all districts in Shenzhen, the current results may somehow reflect the level of HPV infection in women in Shenzhen city.

In summary, the prevalence of HPV infection were various in different age groups and years in women in Shenzhen city. The younger females were at high-risk of HPV infection. The prevention of HPV will be the primary task for women of Shenzhen city in the future.

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