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# HPV genotype analysis for women in Shaanxi Province of China

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**ABSTRACT.** The aim of this study was to examine the subtype distribution of human papilloma virus (HPV) in women in the Shaanxi Province of China. A DNA chip, along with polymerase chain reaction amplification and reverse dot blot technology, was adopted to analyze the HPV genotypes of 22,937 cases of cervical cell specimens. The HPV infection rate was 18.70%, wherein high-risk, low-risk, and high- and low-risk multiple infection rates were 15.75, 2.96 and 1.91%, respectively. High-risk infections accounted for 84.20% of total infections. The rate of HPV infection in women with rural residence, high school education or less, a low income, or age over 40 years was significantly higher than that in the control group (negative HPV infection women). Of the 18 detected high-risk HPV subtypes, the most common in single infections were, in the order of prevalence, HPV16, 58, 18, 52, 33, and 56. For multiple high-risk infections, the most common subtypes in the order of prevalence were HPV16, 52, 58, 18, 56, and 33. Age was a factor in the rate of infection, as the 41-

50-year age group had a significantly higher risk of infection than the other groups ($P < 0.05$). In multiple infections, double infections were common, accounting for 77.10% of multiple infections, and triple or more infections were more common in women aged 51-60 years. In Shaanxi Province, high-risk HPV infection in women was mainly attributed to rural residence, age over 40 years, low income, and low education level.

**Key words:** Age distribution; Human papilloma virus; Subtype distribution; Multiple infection; Cervical cancer

## INTRODUCTION

Cervical cancer is one of the most common malignancies in women worldwide and its incidence ranks second only to breast cancer in China. In recent years, its high occurrence has been primarily found in women aged 45-49 years. The occurrence and development of cervical cancer and cervical human papillomavirus (HPV) infection are closely related. Based on the pathogenic differences, HPV can be divided into high-risk and low-risk categories (Muñoz et al., 2003). The low-risk genotypes do not cause malignant lesions, resulting only in genital warts and low-grade cervical intraepithelial neoplasia grade 1 (CIN1) (Woodman et al., 2001), while the high-risk genotypes are a risk factor for cervical cancer (Sandri et al., 2009). Studies have shown that 99.7% of cancer patients have high-risk HPV infection (Bory et al., 2002). Worldwide, HPV subtypes are significantly different between countries, regions and ethnicities; therefore, understanding the predominant genotypes and epidemiological characteristics of HPV infections in each population can provide guidance for the prevention of cervical cancer in that particular region.

## MATERIAL AND METHODS

### Subjects

#### *General information*

The cervical squamous epithelium cell specimens were collected from female patients who were enrolled in the Shaanxi Provincial Tumor Hospital for treatment, opportunistic screening, health screening, and women's "two cancer" (breast and cervical cancers) screening from January 2011 to May 2014. Selection epidemiologic factors (age, region, education, and average annual income) of cervical lesions were investigated.

#### *Inclusion criteria*

The subjects met the following requirements: resident of a city or county in Shaanxi province, sound intelligence, not pregnant, aged 18 to 65 years, married or with more than 1 year of sexual history, no cervical colonization or history of hysterectomy, no systemic infection or autoimmune diseases, no history of oral immune suppressants, and no vaginal drug or sexual activity 3 days before the gynecological exam. Cervical cancer patients had not undergone surgery, radiotherapy, or chemotherapy.

### *Age distribution*

There were a total of 22,937 cases of women aged 19 to 65 years (mean age, 42.38 ± 20.17 years). From this, 76 women were younger than 20 years, 1033 women were 21 to 30 years old, 4788 women were 31 to 40 years old, 9679 women were 41 to 50 years old, 5298 women were 51 to 60 years old, and 2063 women were older than 60 years.

## Reagents

The HPV genotyping assay kit was provided by Shenzhen Asia Bio Technology Co., Ltd., (China) which simultaneously detects 18 high-risk subtypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 82, and 83) and 5 low-risk subtypes (6, 11, 42, 43, and 81) of HPV. PALL nylon membranes with an aperture of 0.45 mm and a thickness of 6.0 ± 0.5 mils were purchased from the Pall Corporation (New Port Richey, FL, USA).

## Methods

### *Specimen collection and storage*

Colposcopy or vaginal dilator was used to fully expose the cervix, which was wiped with a cotton swab to take a sample of secretions. A cervical brush was then placed in the cervix, spun clockwise 4-5 times and slowly withdrawn to obtain a sufficient sample of cervical epithelial cells. The brush handle was broken off and the brush head was placed into the cervix elution tube, which contained cell preservation solution. The sample was stored at -20°C.

### *DNA extraction*

The cervical brush was thoroughly rinsed and the eluent was transferred into a 1.5-mL tube, which was centrifuged at 13,000 *g* for 10 min, and the supernatant was discarded. The cells were lysed with 50 μL buffer, added to multiple wells, heated at 100°C for 10 min, and immediately centrifuged at 13,000 rpm for 10 min. The middle layer of supernatant contained the DNA solution.

### *Polymerase chain reaction (PCR) amplification*

PCR tubes with 20 μL reaction mixtures were centrifuged at 5000 rpm for 2 s, and then 2 μL mineral oil was added with either 5 μL extracted DNA, blank control (added water), or positive control (HPV positive), resulting in a final reaction volume of 27 μL. Amplification conditions were as follows: 50°C for 15 min, 95°C for 10 min, and then 35 cycles of 94°C for 30 s, 42°C for 90 s, and 72°C for 30 s. A final extension step at 72°C for 5 min completed the reaction.

### *Hybridization, incubation, and blotting*

The samples, film strip (multiple film strips with 23 HPV probes), and all PCR products were added to 5-6 mL solution A (2X saline-sodium citrate, 0.1% SDS) in a 15-mL tube. After denaturation in a boiling water bath for 10 min, the tube was immediately placed

in a 51°C hybridization chamber for 1.5 h. Then the film strips were transferred into 40 mL preheated solution B (0.5X saline-sodium citrate, 0.1% SDS, pH 7.4) and incubated at 51°C for 5 min. The film strips were transferred to POD incubation solution and incubated at room temperature for 30 min. The incubation solution was discarded and the pellet was washed twice for 5 min with solution A at room temperature and solution C (0.1 M sodium citrate) for 2 min. The film strips were placed in freshly prepared colored liquid (19 mL solution C, 1 mL TMB, 10 µL 30% $H_2O_2$) to develop for 15 min in the dark. Last, the film strips were soaked in water and stored in a sealed bag at 4°C. Blue spots were regarded as positive and the presence of particular genotypes was determined by a special analyzer. The positive control was used to monitor the accuracy and reliability of the system.

## Statistical analysis

Data were analyzed using Student *t*-test or nonparametric tests, are reported as means ± standard deviation, and rates were compared with the chi-square test. Multifactor logistic regression analysis was used to analyze the high-risk factors for HPV infection. All analysis used the statistical software SPSS 16.0 (Chicago, IL, USA), with $P < 0.05$ regarded as statistically significant.

# RESULTS

## Demographic characteristics and high-risk HPV infection status

From the 22,937 cervical cell specimens to be genotyped for HPV infection, the average age of the subjects was 41.38 ± 20.17 years; of these subjects, 9408 lived in urban areas and 13,529 lived in rural areas. The general demographic characteristics and rate of high-risk HPV infections are shown in Table 1. A total of 3612 cases had a high-risk infection (including a single high-risk as well as high- and low-risk multiple infections) and the total rate of high-risk infection was 15.75%. Univariate analysis showed that the high-risk HPV infection rate was significantly higher in rural women with high school or lesser education, low-income women, and women over 40 years of age, as compared to the control group ($P < 0.05$).

**Table 1.** Demographic characteristics and high-risk HPV infection status in women.

| Demographics | | Case (N) | Ratio (%) | High-risk HPV infections (%) | P |
|---|---|---|---|---|---|
| Age | ≤40 years | 5,991 | 26.12 | 714 (11.92) | 0.000 |
| | >40 years | 16,946 | 73.88 | 2,898 (17.10) | |
| Region | City | 9,408 | 41.02 | 951 (10.11) | 0.000 |
| | Rural | 13,529 | 58.98 | 2,661 (19.67) | |
| Education | Primary school/illiterate | 3,393 | 14.79 | 634 (18.69) | 0.006 |
| | Middle school | 11,819 | 51.53 | 1,961 (16.59) | 0.003 |
| | College | 7,725 | 33.68 | 1,017 (13.17) | |
| Average annual income | <1,538 (US$) | 14,228 | 62.03 | 2,543 (17.87) | 0.000 |
| | ≥1,538 (US$) | 8,709 | 37.97 | 1,069 (12.27) | |

## Overall status of HPV infection in all age groups

Women aged 41 to 50 years had the highest HPV infection rate ($P < 0.05$; Figure 1), with total and high-risk infection rates of 21.93% (2232/10,177) and 18.64% (1897/10,177),

respectively. The second highest prevalence of HPV infection was the age group of 51 to 60 years, with total and high-risk infection rates of 18.52% (1010/5453) and 16.12% (879/5453), respectively. Women under the age of 20 had the lowest total and high-risk infection rates [5.41% (4/74) and 4.05% (3/74), respectively].

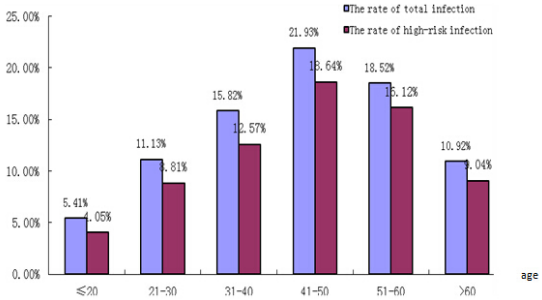


**Figure 1.** Rate of total and high-risk HPV infection in women in all age groups.

## Distribution of HPV genotypes

All 18 high-risk HPV genotypes were detected (Table 2), with HPV16 being the most common one. HPV16 accounted for 40.69% of total single high-risk infection genotypes (1058/2600), followed by HPV58 (9.81%), HPV81 (8.42%), HPV18 (7.08%), HPV52 (6.96%), and HPV43 (5.88%). In multiple infections, the HPV16 subtype was also the most common [16.99% (325/1913)], followed by HPV52 (12.60%), HPV58 (9.57), HPV81 (9.25%), HPV18 (7.95%), and HPV42 (7.58%). The overall detection rate of the HPV16 subtype was 24.39%, which was significantly higher than any other subtype ($P < 0.001$). Other than HPV16, 58, 52, 81, 18, and 42 subtypes were the next most commonly identified genotypes in order of prevalence. HPV16 accounted for 30.64% of high-risk multiple infections (1383/4513), followed by HPV58, 52, 18, 56, and 33, which together accounted for 68.47% (3090/4513) of high-risk multiple infections. Out of the five low-risk genotypes, HPV81 was the most common and accounted for 34.23% (396/1157) of total HPV infections, and it was far more prevalent than the other low-risk subtypes ($P < 0.001$). HPV42 and HPV43 accounted for 82.54% (955/1157) of total HPV infections.

## Distribution of low-risk and high-risk HPV infection in all age groups

In all examined subjects, the rate of single low-risk infection (including single low-risk infection and low-risk subtypes in multiple infections) was 2.96% (678/22,937), accounting for 15.80% (678/4290) of all HPV infections. The prevalence of high-risk subtypes (including single high-risk infection and high-risk subtypes in multiple infection) was as high as 15.75% (3612/22,937), accounting for 84.20% of all HPV infections (3612/4290). The infection rate of high-risk subtypes was significantly higher than that of low-risk subtypes ($P < 0.000$). The rate of high-risk HPV infection in the 41-50 year age group was the highest at 18.64% (1897/10,177), which was significantly higher than that of any other age group ($P < 0.05$). The group of subjects under 20 years of age had only three cases (4.05%) of high-risk infection and 1 case (1.35%) of low-risk infection. The group of subjects over 60 years of age also had low

rates of high- and low-risk infections (9.04% (96/1062) and 1.88% (20/1062), respectively). These data are summarized in Table 3.

**Table 2.** Distribution of HPV genotypes.

| Subtype | | Single infection | Multiple infections | Total | Rate |
|---|---|---|---|---|---|
| High-risk | 16 | 1058 | 325 | 1383 | 24.39 |
| | 18 | 184 | 152 | 336 | 5.93 |
| | 31 | 73 | 99 | 172 | 3.03 |
| | 33 | 128 | 120 | 248 | 4.37 |
| | 35 | 68 | 79 | 147 | 2.59 |
| | 39 | 46 | 42 | 88 | 1.55 |
| | 45 | 30 | 39 | 69 | 1.22 |
| | 51 | 58 | 85 | 143 | 2.52 |
| | 52 | 181 | 241 | 422 | 7.44 |
| | 53 | 93 | 112 | 205 | 3.62 |
| | 56 | 119 | 144 | 263 | 4.64 |
| | 58 | 255 | 183 | 438 | 7.73 |
| | 59 | 80 | 46 | 126 | 2.22 |
| | 66 | 83 | 73 | 156 | 2.75 |
| | 68 | 88 | 116 | 204 | 3.60 |
| | 73 | 22 | 31 | 53 | 0.94 |
| | 82 | 3 | 7 | 10 | 0.18 |
| | 83 | 31 | 19 | 50 | 0.88 |
| Hish-risk total | | 2600 | 1913 | 4513 | 79.60 |
| Low-risk | 6 | 56 | 59 | 115 | 2.03 |
| | 11 | 59 | 28 | 87 | 1.53 |
| | 42 | 142 | 145 | 287 | 5.06 |
| | 43 | 153 | 119 | 272 | 4.80 |
| | 81 | 219 | 177 | 396 | 6.98 |
| Low-risk total | | 629 | 528 | 1157 | 20.40 |
| Overall total | | 3229 | 2441 | 5670 | 100.00 |

Each subtype in the multiple infection was counted separately.

**Table 3.** Distribution of low-risk and high-risk HPV infections in all age groups.

| | Age group (year) | | | | | |
|---|---|---|---|---|---|---|
| | ≤20 | 21-30 | 31-40 | 41-50 | 51-60 | >60 |
| Total case | 74 | 1033 | 5138 | 10177 | 5453 | 1062 |
| Low-risk infection* | 1 | 24 | 167 | 335 | 131 | 20 |
| High-risk infection** | 3 | 91 | 646 | 1897 | 879 | 96 |

*Including low-risk single and multiple infections. **Including high-risk single and multiple infections.

## Single and multiple HPV infection status in different age groups

Of all HPV infections, single HPV infection was most common, with an infection rate of 14.08% (3229/22,937). This accounted for 75.27% of HPV infections (3229/4290), which was significantly higher than the rate of double infection (1061/22,937), which was 4.63% and accounted for 19.07% of HPV infections (818/4290, $P < 0.001$). Single low- and high-risk infection rates were 2.74% (629/22,937) and 11.34% (2600/22,937), respectively, accounting for 14.66% (629/4290) and 60.61% (2600/4290) of total HPV infections, respectively ($P < 0.001$). The rate of double infection was 3.57% (818/22,937), accounting for 19.07% of all HPV infections (818/4290). There were only 49 cases with double infection of low-risk subtypes, while the double high-risk infection and high- and low-risk multiple infection rates were 2.02% (464/22,937) and 1.33% (305/22,937),

respectively, accounting for 10.82% (464/4290) and 7.11% (305/4290) of all HPV infections, respectively. The rate of triple infection was 0.87% (200/22,937), accounting for 4.66% of HPV infection (200/4290). The quadruple infection rate was only 0.19% (43/22,937), accounting for 1.00% of HPV infections (43/4290). In all groups, the single HPV subtype infection rate in the 41-50 age group was 16.64% (1693/10,177). This accounted for 39.46% of all HPV infections (1693/4290), which was significantly higher than all other age groups ($P < 0.05$). These data are summarized in Table 4.

**Table 4.** Single and multiple HPV infection status in different age groups.

| | Age group | | | | | | Total |
|---|---|---|---|---|---|---|---|
| | ≤20 | 21-30 | 31-40 | 41-50 | 51-60 | >60 | |
| Single low-risk | 1 | 21 | 157 | 316 | 114 | 20 | 629 |
| Single high-risk | 3 | 64 | 495 | 1377 | 584 | 77 | 2600 |
| Double low-risk* | 0 | 3 | 10 | 19 | 17 | 0 | 49 |
| Double high-risk | 0 | 9 | 93 | 252 | 101 | 9 | 464 |
| Double multiple | 0 | 9 | 26 | 167 | 96 | 7 | 305 |
| Triple high-risk | 0 | 3 | 12 | 45 | 26 | 2 | 88 |
| Triple multiple | 0 | 6 | 14 | 44 | 47 | 1 | 112 |
| Quadruple high-risk | 0 | 0 | 2 | 3 | 6 | 0 | 11 |
| Quadruple multiple | 0 | 0 | 2 | 1 | 7 | 0 | 10 |
| Five high-risk | 0 | 0 | 1 | 2 | 4 | 0 | 7 |
| Five multiple | 0 | 0 | 1 | 2 | 3 | 0 | 6 |
| Six high-risk | 0 | 0 | 0 | 1 | 1 | 0 | 2 |
| Six multiple | 0 | 0 | 0 | 2 | 3 | 0 | 5 |
| Seven high-risk | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Seven multiple | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| Total | 4 | 115 | 813 | 2232 | 1010 | 116 | 4290 |

*No low-risk was found in low-risk or more multiple infections.

## DISCUSSION

HPV is a member of the Papovaviridae A virus family and is a papilloma vacuole virus. It has closed circular double-stranded DNA with about 8000 base pairs and 3 gene domains (E, L, and UCR). The E domain encodes early proteins, such as E1, E2, E4, E5, E6, and E7, which are mainly related to the biological functions of viral genome replication, transcription regulation, and induction of transformed host cells. The L region encodes L1 and L2 proteins, which are important for viral packaging. The UCR contains the origin of replication and regulatory elements for HPV genomic DNA (Doorbar, 2006). HPV is present in most mammals and birds. Currently there are more than 200 HPV subtypes known, of which more than 30 cause human disease. Different subtypes of HPV can lead to various diseases; the high-risk infections can cause cervical, anal or penile cancer and the low-risk infections cause external genital warts and low-grade cervical intraepithelial lesions (Clifford et al., 2003). An active sex life and poor immune status of underprivileged adult women predispose to HPV infection, and 70 to 80% of women will have at least one HPV infection in their lifetime. The majority of these infections occur in women over the age of 35, when the infection rate may reach 20-50%. Despite the high rate of infection, HPV is usually cleared by the immune system within 8-10 months and only 10-15% of patients will continue to be infected, which could potentially lead to cervical cancer. In China, there is a high rate of HPV infection, with over 18 million new cases each year (Kong and Qu, 2009), and the incidence continues to increase annually. Therefore, timely HPV screening and appropriate early intervention for

cervical lesions is particularly important for cervical cancer prevention and treatment.

In this study, 22,937 women from Shaanxi Province received HPV infection genotyping, in which the total number of infections was 4290 and the total infection rate was 18.70% (4290/22,937). The number of high-risk infections was 3612, with an infection rate of 15.75% (3612/22,937) and accounting for 84.20% of the total infections (3612/4290). This result is consistent with previous reports (Wang et al., 2010; Yang et al., 2012). Statistical analysis showed that high-risk HPV infection in women over 40 years of age from rural areas, with low income and low education level was higher (Table 1). In addition, reports of HPV infection in women from different age groups vary in different parts of China. This study showed that the lowest rates of total and high-risk infection were in the under 20 years of age group (5.41 and 4.05%, respectively), while the 41-50 years age group had the highest rates (21.93 and 18.64%, respectively). This was followed by the 51-60 years age group (total rate was 18.52% and high-risk rate was 16.12%). This is consistent with the high incidence of cervical cancer in the Chinese population, but different from some domestic reports (Li et al., 2010; Dai and Zhang, 2013). It can be speculated that younger women have lesser sexual experience and stronger immune function, thus quickly removing the HPV viral infection, which results in a transient infection. From the 41-50 year age group, many women experienced stress, had an unhealthy lifestyle, relatively frequent sexual activity, and reduced immune function. This hindered immune function could enable the virus to persist, increasing the potentiality of high-risk HPV infection and the susceptibility to long-term infections.

In North America, high-risk HPV infection includes the subtypes HPV16, 53, 52, 18, and 39. In Europe, the common subtypes are HPV16, 18, 31, 33, and 58, and in Africa, the common subtypes are HPV16, 52, 18, 58, and 31 (de Sanjosé et al., 2007). In Tianjin, China, the common high-risk HPV subtypes are HPV16, 58, 52, 33, and 56. In Nanjing, China, the common subtypes are HPV16, 58, 33, 18, and 31, and in Chongqing, China, the common subtypes are HPV16, 58, 52, 18, and 31. Last, in Gansu, China, the common subtypes are HPV16, 58, 52, 31, and 18 (Wang et al., 2010; Yang et al., 2012; Zhang et al., 2013). This study detected 23 subtypes of HPV, from which 18 were high-risk (16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 82, and 83) in women in Shaanxi Province, China. In single HPV subtype infection cases, the most common subtypes were HPV16, 58, 81, 18, 52, and 43, accounting for 47.79% of HPV infections (2050/4290).The most common single high-risk subtypes were HPV16, 58, 52, 18, 56, and 33, which together accounted for 68.47% of infections (3090/4513). In multiple HPV infections, the most common subtypes were HPV16, 52, 58, 81, 18, and 42, accounting for 50.10% (1223/2441) of all HPV infections. HPV16 had an overall detection rate of 24.39%, which is significantly higher than that of other HPV subtypes ($P < 0.001$). Distribution of low-risk subtypes in HPV infection in all age groups was consistent with the distribution of high-risk HPV infections (Table 3). We detected five low-risk subtypes of HPV, and the most common single low-risk subtypes were HPV81, 43, 42, 11, and 6, accounting for 14.66% of HPV infections (629/4290). The most common low-risk subtypes in multiple infections were HPV81, 42, 43, 6, and 11, accounting for 6.98% of multiple infections (396/5670). Low-risk HPV infections in China are mostly reported to be caused by HPV6 and 11 (Lee et al., 2003; Wang et al., 2010). In Lee's study, HPV81 was the most common low-risk subtype in single (34.82%, 219/629) and multiple infections (33.52%, 177/528), which has also been reported in Chongqing, China (Kong and Qu, 2009; Wang et al., 2010). The most prevalent subtypes in low-risk single infections were HPV81, 43, 42, 11, and 6, and the most prevalent subtypes in low-risk multiple infections were HPV81, 42, 43, 6,

and 11 (Table 2). The results reported in this study do show some discrepancies from reports in other areas of China (Wang et al., 2010; Jiang et al., 2012).

This study showed that HPV infection has a clear regional character, meaning that different countries and regions have their own unique subtype distribution characteristics. Numerous studies have demonstrated the epidemiological and molecular biology of HPV and have shown that HPV infection can be prevented by a vaccine, which can also greatly reduce or even eliminate cervical cancer. HPV infection has obvious geographical features, which creates pressure to make a vaccine that targets all prominent subtypes, and targeted research and development for a vaccine with region-specific subtypes will result in lower levels of cervical cancer. Therefore, knowledge regarding the distribution of HPV subtypes in a given region has great value for screening, prevention, treatment, and vaccine development for cervical cancer.

Single HPV infection is about three times more common than multiple infections (Jiang et al., 2012). In Jiang et al. (2012)'s study, a single HPV infection accounted for 75.27% (3229/4290) of the total infections, and the HPV16 subtype was the most common (24.66%, 1058/4290). The rate of multiple infection was 24.73% (1061/4290), resulting in a ratio of four single infections to one multiple infection, which is slightly higher than that in previous reports (Jiang et al., 2012) (Table 3). In multiple infections, the most common combination was HPV16 with other subtypes (13.31%, 325/2441), followed by HPV52, 58, 81, 18, and 42. Multiple infections accounted for 40.23% (982/2441) of all infections (Table 2). Studies have shown that the risk of cervical cancer can be increased nearly 20 times with a single HPV infection and 32 times with multiple HPV infections. Compared to single infection, multiple HPV infection results in a higher viral load, leading to a higher risk of cancer, faster lesion development, and higher recurrence (Lee et al., 2003; Fan et al., 2009; Zhao et al., 2009). It is suggested that, when focusing on single clinical infections, regular follow-up and monitoring of multiple HPV infections should be performed to reduce the incidence of cervical cancer.

In different age groups, the highest risk of infection was noted in the 31-50 years group, followed by the 51-60 years and 21-30 years groups, with age £20 years and age >60 years having the lowest risk ($P < 0.05$). The risk of infection was the highest in the 41-50 years age group, followed by the 51-60 years (16.12%) and the 31-40 years age groups (12.57%). The risk of infection was lowest in the £20 yearsage group (4.05%) (Table 3). The infection rate for single low-risk infection was 14.66% (629/4290), and single high-risk infections accounted for 60.61% (2600/4290) of total infections. The majority of these infections were double high-risk and double multiple infections (94.01%, 769/818). For low-risk multiple infections, there were only double low-risk subtype infections and infection with three or more subtypes was not observed (Table 4). For infection with four or more multiple subtypes, there were only 43 cases, accounting for 1.00% of all infections (43/4290) and 1.76% of multiple infections (43/2441). The rate of high- and low-risk multiple infections was 1.91% (439/22,937), accounting for 10.23% of total multiple infections (439/4290) (Table 4).

In different age groups, a single infection was still the most common form of infection, followed by superinfection. For a single HPV infection, the infection rate in the 41-50 years age group was 16.64% (1693/10,177) and high-risk single infection rate was 13.53% (1377/10,177), which was significantly higher than that in the other groups ($P < 0.05$). In women who were 31-60 years old, the double infection rate was similar. In women under20 years of age, there was only one case of single infection and no multiple infections. In the 21-30 years and >60 years age groups, one case with four or more subtypes was found. Multiple

infections with six or seven subtypes only occurred in the 41-60 years age group.

The analysis for infection with three or more subtypes showed that the 51-60 year age group had the highest rate at 1.79% (98/5453), accounting for 9.70% of total infections (98/1010), which was significantly higher than that for the other age groups ($P < 0.05$). This result shows that the prevalence of multiple infection increases with age. After 60 years of age, a significant reduction was found in multiple infections (Table 4). The women over 40 years of age had a high incidence of cervical cancer. It takes about 5-15 years to progress from HPV infection to cervical cancer; therefore, women who are 30-60 years old should be primary subjects for HPV screening for reducing the incidence of cervical cancer.

In Europe and other developed countries, the HPV test has been regarded as one of the preferred screening methods for prevention of cervical cancer because it has much higher sensitivity than cytology, making it important for primary screening, detection, and treatment (Garcia-Echeverria and Sellers, 2008). As a result, the incidence and mortality of cervical cancer in Europe and America have significantly reduced after the effective implementation of screening. In China, similar measures should be taken in cervical cancer screening for women to enhance cancer health education, such as explanation of risk factors and early signs of cervical cancer. Better screening methods for HPV need to be developed in order to detect lesions before the occurrence of cervical cancer, thus achieving early diagnosis and treatment. In addition, development of region-specific HPV vaccines based on epidemiological data will effectively reduce the incidence of cervical cancer.

## Conflicts of interest

The authors declare no conflict of interest.

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