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Prevalence and genotype distribution of human papillomavirus in women in the Henan Province

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ABSTRACT. We studied human papillomavirus (HPV) prevalence and genotype distribution among women in the Henan Province to provide epidemiological data as a means of preventing cervical cancer and developing a vaccine. A total of 14,873 samples were genotyped by using polymerase chain reaction reverse dot-blot. The overall HPV-positive rate in the sample was 23.98% (3566/14873), of which 69.01% (2461/3566) were infected with high-risk HPV types and 17.33% (618/3566) with low-risk types. Eighteen high-risk HPV types were detected; HPV 16 (16.73%) was the most common, followed by 58 (10.17%), 52 (9.11%), 56 (6.48%), 66 (5.76%), 33 (4.74%), 68 (3.92%), 31 (3.60%), 53 (3.13%), 59 (3.00%), 35 (2.53%), 51 (2.00%), 73 (1.08%), 45 (0.94%), 83 (0.84%), 39 (0.69%), 18 (0.61%), and MM4 (0.04%). Four low-risk HPV types were detected; HPV 43 (11.34%) was the most common, followed by 6 (5.17%), 42 (4.76%), and 11 (3.35%). Type 44 was not detected. Among the women positive for HPV, 71.17% (2538/3566) had a single type of infection; of these, 54.66% (1949/3566) had high-risk and 16.52% (589/3566) had low-risk infections. A total of 28.83% (1028/3566) had multiple HPV infec-

tions, of which 20.11% (717/3566) had double HPV infections. One peak in HPV prevalence occurred among women younger than age 25; a second peak occurred among women older than age 55. The overall prevalence of HPV infection in the Henan Province was 23.98%, of which the most common type was high-risk HPV and a single type of infection. The leading genotypes were HPV 16, 43, 58, 52, and 56.

Key words: Human papillomavirus; Genotypes; Vaccine; Cervical cancer

INTRODUCTION

Epidemiology and basic research have confirmed that human papillomavirus (HPV) infection is a major cause of cervical intraepithelial neoplasia and cervical cancer (Pierce Campbell et al., 2012; Ward et al., 2012). HPV infection has a strong regionalism, and HPV infection rates and distribution in different countries or regions are different (Clifford et al., 2005); therefore, studying the distribution of HPV in a particular region provides important guidance for developing and applying vaccines. To understand the infection status and distribution characteristics of HPV in women in the Henan Province, we undertook HPV genotyping in 14,873 women in the Province to provide a theoretical basis for prevention and treatment of cervical cancer and development of HPV vaccines.

MATERIAL AND METHODS

Research subjects

The database consisted of 14,873 women from the outpatient services of the departments of gynecology, reproductive medicine, women's health, and the health physical of The Third Affiliated Hospital of Zhengzhou University between February 2012 and May 2013. Inclusion criteria were women who had lived in the Henan Province for more than 5 years and were sexually active. Participants' ages mainly ranged from 25 to 45 years, with a median age of 36 years; the youngest was 14 and the oldest was 88.

Main reagents and equipment

An HPV genotyping detection kit [polymerase chain reaction (PCR)/reverse dot blot] reading instrument was used (Yaneng Bio Technology Co., Ltd.). It can detect 18 high-risk subtypes: HPV 16, 18, 31, 33, 35, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 83, and MM4, and 5 kinds of low-risk subtypes: HPV 6, 11, 42, 43, and 44.

Experimental method

Draw materials

We placed the specific sampling brush for HPV into the patient's cervix, uniaxially rotated the brush 4 to 5 times, put the brush head into the elution tube, broke the brush handle off, tightened the lid, and made marks.

DNA extraction

We ensured the brush was eluted sufficiently and then wiped it, transferring 1 mL of eluent into a 1.5-mL centrifuge tube. The tube was centrifuged for 10 min at 13,000 revolutions per min (rpm); we then discarded the supernate and retained the precipitated cells in the bottom of the tube. We then added 50 µL of lysate, suspended the sedimentation, placed it in a boiling water bath for 30 min, centrifuged it for 10 min at 13,000 rpm, and retained the supernate for later use.

PCR amplification

We took the PCR reaction tube, centrifuged it for 2 s at 5000 rpm, and added 5 µL extracted DNA. A reaction system of 25 µL was amplified under the following conditions: 50°C for 15 min; 95°C for 10 min; 94°C for 30 s; 42°C for 90 s; 72°C for 30 s; 40 cycles; 72°C for 5 min.

Hybridization

We took a 15-mL plastic centrifuge tube, added 5-6 mL of liquid A and 25 µL of the PCR product, placed it in a boiling water bath for 10 min, and hybridized it for at least 1.5 h in a hybrid box at 51°C.

Membrane wash

We removed the membrane strip to a 50-mL tube containing preheated solution B, which was shaken gently and washed for 5 min at 51°C.

Color reaction

We removed the membrane strip, placed it into the incubation medium containing liquid A and POD (2000:1), shook it gently, and incubated it for 30 min at room temperature, after which the incubation medium was discarded. The membrane strip was then shaken and washed with liquid A at room temperature twice, each time for 5 min. We washed the membrane with liquid C for 1-2 min at room temperature while the color liquid was being prepared. The membrane strip was then dipped into a color-substrate solution to dark color for 30 min in dark. We read the HPV genotyping results with the previously mentioned reading instrument.

Statistical analysis

Data were analyzed using the SPSS 17.0 statistical software. F and chi-square tests were compared in every group. A P value <0.05 was considered to be statistically significant.

RESULTS

Detection of the genotype of HPV infection

The overall HPV-positive rate in women was 23.98% (3566/14,873). Of the 23 sub-

types, 22 were measured; the low-risk HPV 44 type was not measured. From the frequency of detected subtypes, high-risk HPV genotypes accounted for 75.38% of samples (3690/4895), with HPV rankings from high to low as follows: 16 (16.73%), 58 (10.17%), 52 (9.11%), 56 (6.48%), 66 (5.76%), 33 (4.74%), 68 (3.92%), 31 (3.60%), 53 (3.13%), 59 (3.00%), 35 (2.53%), 51 (2.00%), 73 (1.08%), 45 (0.94%), 83 (0.84%), 39 (0.69%), 18 (0.61%), and MM4 (0.04%). The low-risk HPV phenotypes accounted for 24.62% of samples (1205/4895), with the HPV rankings from high to low as follows: 43 (11.34%), 6 (5.17%), 42 (4.76%), 11 (3.35%), and 44 (0.00%) (the low-risk HPV 44 genotype was not measured). The comprehensive rankings of high-risk and low-risk were as follows: 16 (16.73%), 43 (11.34%), 58 (10.17%), 52 (9.11%), 56 (6.48%), 66 (5.76%), 6 (5.17%), 42 (4.76%), 33 (4.74%), 68 (3.92%), 31 (3.60%), 11 (3.35%), 53 (3.13%), 59 (3.00%), 35 (2.53%), 51 (2.00%), 73 (1.08%), 45 (0.94%), 83 (0.84%), 39 (0.69%), 18 (0.61%), MM4 (0.04%), and 44 (0.00%). For the distribution of the 14,873 HPV infection cases, see Table 1 and Figure 1.

Table 1. Distribution of HPV infection.

Groups	HPV subtype	Cases (N)	Proportion (%)
High-risk	16	819	16.73
	18	30	0.61
	31	176	3.60
	33	232	4.74
	35	124	2.53
	39	34	0.69
	45	46	0.94
	51	98	2.00
	52	446	9.11
	53	153	3.13
	56	317	6.48
	58	498	10.17
	59	147	3.00
	66	282	5.76
	68	192	3.92
	73	53	1.08
	83	41	0.84
	MM4	2	0.04
	Total	3690	75.38
Low-risk	6	253	5.17
	11	164	3.35
	42	233	4.76
	43	555	11.34
	44	0	0.00
	Total	1205	24.62

HPV, human papilloma virus in mixed infections, each subtype was individually counted. A total of 4895 subtypes were determined.

Single and multiple HPV infections

In 3566 cases of infected women, high-risk infection accounted for 69.01% of infections (2461/3566), low-risk infections accounted for 17.33% (618/3566), and mixed HPV infection accounted for 13.66% (487/3566).

In 3566 cases of infected women, more than 1 HPV infection (2538 cases) accounted for 71.17% of infections (2538/3566), in which high-risk infection accounted for 54.66% (1949/3566) and low-risk infection accounted for 16.52% (589/3566). A total of 1028 cases of multiple HPV infections accounted for 28.83% (1028/3566), in which double infection ac-

counted for 20.11% (717/3566). Of the high-risk double infection cases, the numbers significantly decreased as the number of genotypes increased. Eight particular HPV subtypes were simultaneously detected in multiple infections the most often (Table 2).

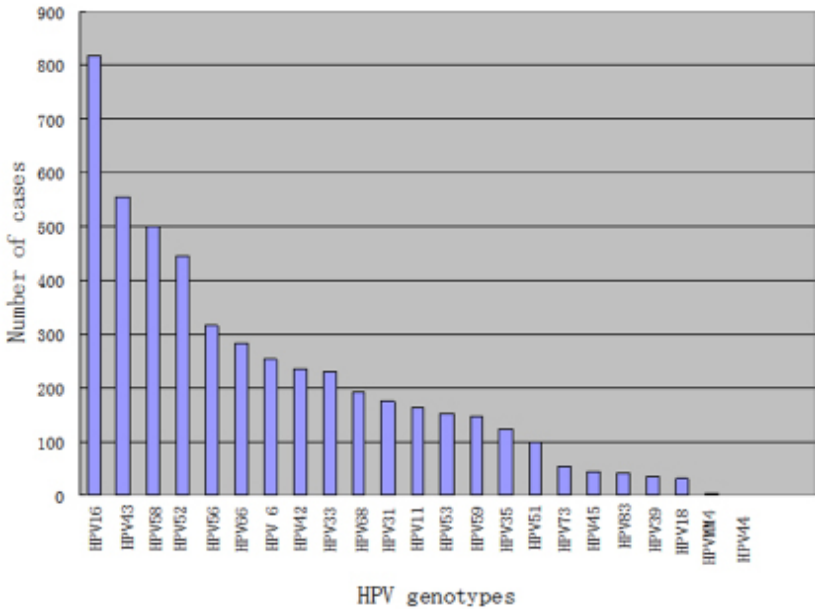

Figure 1. Distribution of human papilloma virus genotypes in the Henan province.

Table 2. Distribution of types of human papilloma virus infection [N (%)].

Type of infection	No. of cases	Low-risk	High-risk	Mixed
Single	2538 (71.17%)	589 (16.52%)	1949 (54.66%)	-
Double	717 (20.11%)	26 (0.73%)	414 (11.61%)	277 (7.77%)
Triple	198 (5.55%)	3 (0.08%)	72 (2.02%)	123 (3.45%)
Quadruple	63 (1.77%)	0 (0.00%)	21 (0.59%)	42 (1.18%)
Quintuple	34 (0.95%)	0 (0.00%)	5 (0.14%)	29 (0.81%)
Sextuple	12 (0.34%)	0 (0.00%)	(0.00%)	12 (0.34%)
Septuple	2 (0.06%)	0 (0.00%)	(0.00%)	2 (0.06%)
Octuple	2 (0.06%)	0 (0.00%)	(0.00%)	2 (0.06%)
Total	3566 (100%)	618 (17.33%)	2461 (69.01%)	487 (13.66%)

Age distribution of HPV infection

Research participants were divided into 5 groups, and HPV infection rates showed an approximate U-shaped curve in terms of age. Two peaks were formed: the first HPV infection peak occurred before age 25; the second peak occurred in women older than age 55 (Figure 2); see Table 3 for the rate of HPV infection. The group that was younger than 25 years of age showed a higher HPV infection rate than the other age groups ($\chi^2 = 22.747$, $P = 0.000 < 0.05$), There was no significant difference in the other age groups ($\chi^2 = 6.759$, $P = 0.08 > 0.05$).

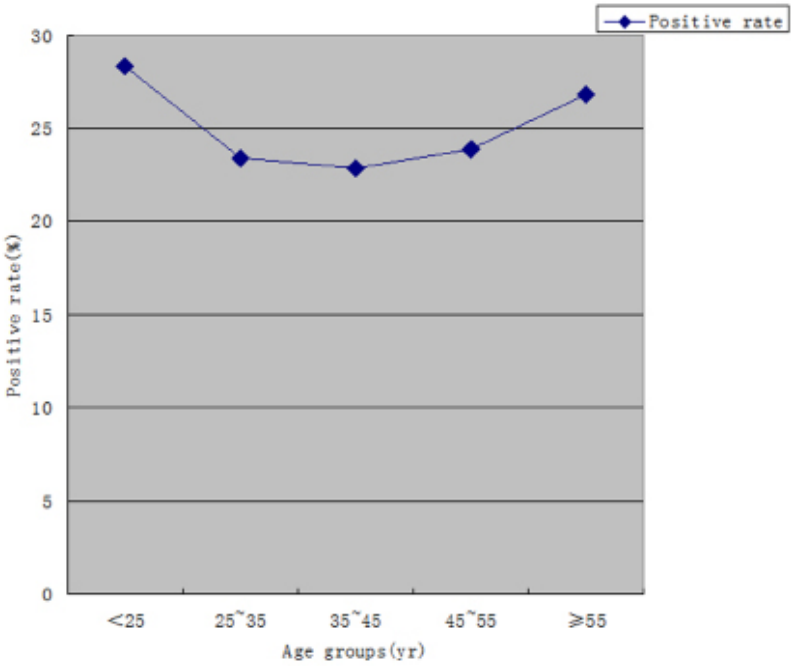

Figure 2. U-shaped relationship between age group and human papilloma virus infection rate.

Table 3 . Age distribution of HPV infection.

Age groups (years)	Cases (N)	Negative cases (N)	Positive cases (N)	Positive rate (%)
<25	1413	1013	400	28.31
25-35	5318	4073	1245	23.41
35-45	4957	3823	1134	22.88
45-55	2298	1749	549	23.89
≥55	887	649	238	26.83[a]
Total	14873	11307	3566	23.98

Compared 5 groups of infection rates: $\chi^2 = 22.747$, $P = 0.00 < 0.05$. Compared 4 groups of infection rates of women older than 25 : $\chi^2 = 6.759$, $P = 0.080 > 0.05$. Compared the group of women younger than 25 and women older than 55, $\chi^2 = 0.593$, [a]$P = 0.441 > 0.05$.

DISCUSSION

Cervical cancer is the second most common cancer and seriously threatens women's health. Currently, it also is the only cancer that has a definite cause that can be treated early and prevented – and then eradicated. High-risk HPV persistent infections are the main causes of cervical cancer and precancerous lesions (Pierce Campbell et al., 2012; Ward et al., 2012). Another study reported positive HPV infections in up to 99.7% of cervical cancer tissue specimens (Miura et al., 2006). In 2008, about 529,800 new cases of cervical cancer appeared, 275,000 women died from the disease worldwide, and it was predicted that the figure would likely rise to 410,000 cases in 2013 (Motoyama et al., 2004).

Currently, HPV has more than 200 identified genotypes, in which at least 42 are connected with reproductive tract infections (Muñoz et al., 2003). Epidemiological data showed

that the pathogenicity of different HPV types had significant differences, such as some types of HPV being closely related to cervical cancer because persistent infection can lead to lesion progression and ultimately to cervical cancer (Motoyama et al., 2004). Therefore, some scholars believe that these potentially carcinogenic HPV genotypes can be important detection indices in the early stages of infection (Motoyama et al., 2004) and can provide the basis for early diagnosis, adequate clinical solutions, and further treatment protocols. In February 2005, the International Agency for Research on Cancer presented the differences in carcinogenicity from various types of HPV that should be considered when detecting cervical cancer resulting from HPV (Cogliano et al., 2005). In addition, clear regional distribution genotypes of HPV are significant for research and development of an HPV vaccine and prevention of cervical cancer.

Current screening for cervical cancer is carried out through cytological detection and HPV-DNA detection; both of these methods are means of secondary prevention. These methods screen out patients with early cervical lesions or cervical cancer patients at high risk as a means of early diagnosis and early cure. The most effective and safe measures should be primary prevention and a prophylactic HPV vaccine. The vaccine currently being used in clinical practice in the West has an efficient immune activity and can produce higher antibody titers to reduce persistent HPV infection and related clinical disease, thereby reducing cervical cancer. However, China is still in the developmental stages of such a vaccine and has not yet entered HPV clinical trials. China's broad geographical expanse, large population, and whether genotypes of regional HPV infection are different means there is still a need to improve epidemiological data. Developed countries generally use a bivalent (16/18) or tetravalent (16/18/6/11) HPV vaccine (Yoshikawa, 2009), with HPV 16/18 being the most common. However, according to existing epidemiological data, these vaccines do not cover the main HPV genotypes in specific areas of China. Therefore, understanding the trends of HPV occurrence in different regions and populations and developing the HPV vaccine that is appropriate for women in China will be an important step toward preventing cervical cancer in the future.

A large amount of epidemiological data shows that infection status and distribution of HPV are significantly different in different countries or regions. Clifford et al. (2005) found that the most common infection type of HPV around the world is HPV 16, followed by HPV 42, 58, 31, 18, 56, 81, 35, 33, and 45. Ranking second and third in Asia were HPV 33 and HPV 56, and ranking second in South America was HPV 58, whereas in Europe it was HPV 31. Rui et al. (2012) reported that the main types of infection were HPV 16, 18, 58, 56, and 52 in East Asia; HPV 52, 16, 58, 18, and 66 in Southeast Asia; and HPV 16, 18, 45, 33, and 35 in South Asia (Bhatla et al., 2008). The main types of infection in France were HPV 16, 31, 33, 45, 52, and 58 (Monsonego et al., 2012); in Korea, HPV 16, 52, 58, 18, and 56 (Lee et al., 2012); and in Taiwan, HPV 52, 16, 56, and 18 (Chen et al., 2011).

The present study showed that the main types of high-risk HPV infection in the Henan Province were HPV 16, 58, 52, 56, and 66, whereas HPV 18 showed a low infection rate. The lowest risk type was HPV 43, followed by 6, 42, and 11. HPV 44 was not found in the Province. The most common low-risk infection was HPV 16, followed by 43, 58, 52, 56, and 66. The low-risk HPV 43 had a very high infection rate in the Henan Province, ranking second only to 16. The results of our study were different from reported results in other areas of the country. The main types of infection in Tibet have been reported to be HPV 16, 33, 58, 52, and 31 (Jin et al., 2009). The top 5 HPV types were reported in the following Provinces: Liaoning, HPV 16, 18, 58, 33, and 31 (Liu et al., 2010); Shandong, HPV 16, 52, 58, 18, and 11 (Liu et al., 2007); Guangxi, HPV 52, 16, 58, 53, and CP8304 (Li et al., 2012); and Zhejiang, HPV

52, 16, 58, 68, and 81 (Ye et al., 2010). However, the top 6 types in Hunan were HPV 16, 52, 58, 18, 6, and 39 (Li et al., 2013). These studies show that the distribution of HPV in various regions has regional differences, although HPV 16 plays a dominant role in all regions, but the importance of the other subtypes in different regions vary. To be effective in preventing HPV infection, investigating the prevalent HPV types in various regions and developing an HPV vaccine that works for these regions is necessary.

The present study also investigated 14,873 Henan women; we found 3566 cases of HPV infection, with infection rates as high as 23.98%. High-risk infection accounted for 69.01% of cases and low-risk infection accounted for 17.33%; low-risk mixed with high-risk infections accounted for 13.66% of cases. HPV infection in Henan was significantly higher than in Zhejiang (13.3%) (Ye et al., 2010) and Taiwan (16.2%) (Chen et al., 2011), but is similar to that of Changchun (Liu et al., 2013), Hunan (Li et al., 2013), and Chaozhou in Guangdong (Chen et al., 2012), where the infection rates were 20.0, 22.6, and 24.5%, respectively, but below that of Guangxi (38.9%) (Li et al., 2012).

Mixed HPV infection is more common in molecular epidemiology of cervical cancer because certain HPV genotypes interact or have synergistic interactions that promote the development of cervical lesions (Trottier et al., 2006). In particular, the risk that occurs in high-grade lesions and invasive cancers in multiple HPV infections is greater than in a single infection (van der Graaf et al., 2002). Compared with a single HPV infection, multiple infections increase the risk of precancerous lesions (van der Graaf et al., 2002). Herrero et al. (2005) found that when HPV 16 is mixed with other types of infection, the risk of cervical lesions could greatly increase. Lee et al. (2003) further studied the relationship between multiple infections and cervical cancer and found that although a single infection increased the risk of suffering from cervical cancer by 19.9 times, multiple infections increased the risk by 31.8 times. Therefore, women with multiple infections should increase scrutiny to prevent the development of cervical cancer. The results showed that a single infection (2538 cases) occurred most often in the 3566 cases, accounting for 71.17%; 1028 cases of multiple infections accounted for 28.83%, of which double infection accounted for 20.11%. Eight HPV subtypes were the most simultaneously detected in a multiple infection. Although the Henan Province has more single infections, multiple infections account for about a third of all infected women.

General studies suggest that high HPV infection rates in young women are related to being sexually active. As mentioned previously, the present study found that HPV infection rate showed an approximate U-shaped curve in terms of age: the infection rate of women younger than 25 years was significantly higher than in other age groups ($\chi^2 = 22.747$, $P = 0.000 < 0.05$) and the infection rate in women older than 55 years also showed an increase. These results are similar to the outcomes of Yip et al. (2010) and Lee et al. (2012). As a means of analyzing the reasons for these outcomes, it may be that young women have a more active sex life; HPV infection is closely related to sexual activities. This study showed that the first peak of HPV infection occurred before age 25 years; the HPV infection rate of women older than 55 years may be associated with low immunity. The present results suggested that early sex education for young people in the Henan region should be increased, and cervical cancer screening of older women also should receive increased attention.

This study analyzed genotype distribution characteristics of HPV infection in women of the Henan Province; it was determined that HPV 16, 43, 58, 52, and 56 are the most common types of infection in the Province. If an HPV vaccine can contain these types, it will be effective in preventing HPV infection and reducing the occurrence of cervical intraepithelial

neoplasias or cervical cancer in women in the Henan Province. At present, the relevant genotype distribution of HPV in the Province has not been studied in a large sample size, but we believe this study can provide an epidemiological basis for the development of an HPV vaccine in the Henan Province.

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