

## RESEARCH ARTICLE

# Prevalence and Genotype Distribution of Human Papillomavirus among Women from Henan, China

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### Abstract

Human papillomavirus (HPV) infection has been implicated as a causative of cervical cancer. In the present study, a total of 578 samples from females attending the gynecological outpatient clinic in Henan province, China, were collected and the HPV genotypes were detected by gene chip and flow-through hybridization. Overall, 44.5% (257/578) females were found to be HPV DNA positive, and the high risk HPV (HR-HPV) rate was 35.1% (203/578). The first peak of HR-HPV infection appeared in the >60 year-old group (55.0%), and the second was within the 51-55 year-old group (50.0%) ( $\chi^2=19.497$ ,  $p<0.05$ ). HPV 16 was the most prevalent genotype (9.2%), followed by HPV 52 (7.8%), HPV 6 (6.9%), HPV 11 (5.9%) and HPV 42 (5.0%). The single type HPV infection was 30.4%, with the five majority prevalent genotype HPV 16 (16.5%), HPV 52 (14.3%), HPV 6 (12.6%), HPV 42 (8.6%), HPV 31 (5.1%). The multiple-type HPV infections were 14.0%, and HPV 16 was the most prevalent type (29.6%), followed by HPV 52 (24.7%), HPV 6 (22.2%), HPV 11 (22.2%), HPV 42 (17.3%) and HPV 39 (17.3%).

**Keywords:** Human papillomavirus - prevalence - genotype - Henan, China

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### Introduction

Cervical cancer is the third most commonly diagnosed cancer and the fourth leading cause of cancer death in females worldwide (Jemal et al., 2011). More than 80% of the women diagnosed with cervical cancer live in the developing countries, such as China and Indian, the cervical cancer has been an important public health concern (Li et al., 2013; Rai et al., 2014). Several methods, such as visual inspection using acetic acid or Lugol's iodine, the conventional Pap smear and human papillomavirus (HPV) DNA testing, had been implemented for the screen of cervical cancer. The sensitivity of visual inspection and Pap smear might depend on the experience and training of the health workers, however, HPV DNA testing would be an alternative method for objectively screening HPV infection with high sensitivity and moderate specific (Zhao et al., 2010).

HPV infection has been proven to be the etiologic agent of cervical cancer (Ferlay et al., 2010). It is reported that more than 200 different HPV genotypes have been identified, and about 40 oncogenic subtypes are associated with the majority of cases of cervical cancer (Munoz et al., 2003). Although most of the HPV infections are transient and may resolve spontaneously, the persistent infections with subset of HPV genotypes are necessary for the development of cervical cancer and its precursors (Schlecht et al., 2001). Based on their association with

cervical cancer, HPV genotypes can be classified into high-risk HPV (HR-HPV, consisting of HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59) and low-risk HPV (LR-HPV, including HPV 6, 11, 40, 42, 43 and 44) (Munoz et al., 2003). To some extent, the detection of HR-HPV may reduce the risk of developing cervical cancer and associated deaths (Levin et al., 2010). It is reported that the distribution and prevalence of HPV varied by geographic regions, even in different areas of the same country (Wheeler et al., 2009). Although a series of studies have been conducted to assess the prevalence of HPV genotypes in China, such as Shanghai, Zhejiang and, Shenzhen, fewer are referred to Henan province (Xue et al., 2009; Ye et al., 2010; Wang et al., 2013).

The principal objective of the present study is to investigate the prevalence and distribution of HPV genotypes among females from a hospital in Luoyang, Henan, by means of the commercially available HPV DNA Chip.

### Materials and Methods

#### Sample Collection

From December 2008 to January 2014, consecutive samples of 578 cervical swabs from women attending the gynecological outpatient clinic were collected by healthcare workers in No. 150 Central Hospital of PLA.

Before collection, the woman was eligible to be in

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the study if she: (a) had not had sexual intercourse in the previous 48 hours; (b) had no use of vaginal medication or washing in the previous 48 hours; (c) had no use of acetic or iodine; (d) was not presently during menstruation; (e) was mentally and physically competent.

All the specimens and their corresponding clinical information were obtained under protocols approved by the Ethics Committee of the hospital, and the written informed consents were obtained from all the patients.

Every sample was obtained with a cytobrush from the ecto- and endocervix of the uterus of each woman, and then suspended in the physiologic saline and stored at -70°C until the HPV genotyping.

### HPV Genotyping

After thrown, the supernatants were removed by centrifugation at 14000 rpm for 5 minutes and the pellets were collected for DNA extraction. Genomic DNA was extracted by alkalysis using DNA extraction kit (Chaozhou Hyribio Biotechnology Corp., China). The quality of the extracted DNA was confirmed by amplifying the  $\beta$ -globin gene as an internal control. The HPV DNA was amplified using the PCR kit (Chaozhou Hyribio Biotechnology Corp., China) with the general consensus primers PGMY09/PGMY11 to amplify the HPV L1 gene. According to the recommendation of the manufacturer, PCR was carried out in 25  $\mu$ L reaction mixture per person in a thermal cycle (PE Applied Biosystems GeneAmp PCR System 9600) following parameters: initial denaturation at 95°C for 9 min, followed by 40 cycles at 95°C for 20s, 55°C for 30s, and 72°C for 30s, and a final extension at 72°C for 5 minutes. Distilled water and HPV 18 were presented separately as negative and positive control.

HPV genotyping was detected by flow-through

hybridization and gene chip by HybriMax (Chaozhou Hyribio Limited Corporation, Chaozhou, China) according to the manufacturer's instruction. The chip can determine 21 subtypes, including 5 LR-HPV (namely 6, 11, 42, 43, 44), 16 HR-HPV (16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68 and CP8304). The genotyping was performed via hybridization of the PCR products to gene chip containing genotype-specific oligonucleotides that were immobilized on a nylon membrane. The amplicon was subsequently denatured at 95°C for 5 minutes, and then kept at 45°C for 10 minutes for hybridization on the platform of hybridization chamber (HMM2). After blocking with confining fluid, the chips were washed 3 times, and then the hybrids were detected by the addition of streptavidin-horse-radish peroxidase conjugate which binded to the biotinylated PCR products and incubated at 25°C for 3.5 minutes. After 4 times washing, the substrate NBT/BICP (nitroblue tetrazolium and 5-bromo-4-chloro-3-indoylphosphate) was added at 37°C for 5 minutes, followed by 4 times washing. The final results were detected by colorimetric change on the chip under direct visualization.

### Statistical Analysis

SPSS version 19.0 (IBM, Armonk, NY, USA) was used to assess the significance of differences detected in the frequency of HPV infections among groups. The  $\chi^2$  test was used to compare the prevalence of HPV infection. A *p*-value <0.05 was considered statistically significant.

## Results

### Prevalence of HPV Infection in Different age Groups

A total of 578 samples from females (ranging from

**Table 1. Prevalence of HPV Among Different Age Groups (n=578)**

Category, HPV type	Sample numbers	Overall prevalence (100%)	Prevalence by age group (100%)									
			$\leq 20$ (n=14)	21-25 (n=81)	26-30 (n=72)	31-35 (n=75)	36-40 (n=107)	41-45 (n=94)	46-50 (n=69)	51-55 (n=28)	56-60 (n=18)	>60 (n=20)
Any HPV type	257	44.5	71.4	42	55.6	33.3	46.7	38.3	33.3	57.1	50	65
Low risk types	97	16.8	35.7	18.5	19.4	8	15	18.1	10.1	28.6	11.1	35
6	40	6.9	28.6	4.9	12.5	1.3	9.3	5.3	2.9	10.7	5.6	5
11	34	5.9	7.1	7.4	6.9	2.7	4.7	4.3	2.9	14.3	0	25
42	29	5	0	6.2	1.4	5.3	1.9	9.6	7.2	7.1	0	5
43	2	0.3	0	0	1.4	0	0	0	0	0	5.6	0
6	6	1	0	2.5	0	1.3	1.9	0	0	0	0	5
High risk types	203	35.1	42.9	30.9	47.2	30.7	38.3	24.5	27.5	50	38.9	55
16	53	9.2	14.3	6.2	8.3	4	8.4	8.5	13	10.7	16.7	25
18	12	2.1	7.1	1.2	1.4	0	1.7	0	1.4	14.3	0	10
31	18	3.1	0	3.7	1.4	1.3	5.6	1.1	2.9	7.1	0	10
33	9	1.6	0	1.2	1.4	0	0.9	2.1	4.3	0	0	5
35	4	0.7	0	0	0	0	0.9	2.1	1.4	0	0	0
39	22	3.8	7.1	3.7	6.9	4	3.7	2.1	2.9	3.6	0	5
45	2	0.3	0	1.2	0	0	0	0	0	0	0	5
51	7	1.2	0	1.2	0	0	2.8	0	2.9	0	0	5
52	45	7.8	7.1	9.9	11.1	8	9.3	4.3	4.3	7.1	5.6	10
53	20	3.5	14.3	3.7	5.6	8	0.9	0	1.4	0	5.6	10
56	11	1.9	7.1	2.5	1.4	2.7	0.9	1.1	0	3.6	0	10
58	22	3.8	21.4	4.9	4.2	0	2.8	1.1	2.9	7.1	5.6	15
59	15	2.6	0	3.7	1.4	6.7	2.8	0	0	0	5.6	10
66	10	1.7	0	2.5	0	0	0.9	2.1	0	10.7	0	10
68	16	2.8	7.1	2.5	2.8	2.7	0.9	2.1	2.9	14.3	0	0
CP8304	15	2.6	0	2.5	2.8	2.7	0	5.3	4.3	0	0	5
Single infection	176	30.4	64.3	23.5	43.1	20	34.6	28.7	21.7	32.1	50	20
Mutiple infections	81	14	7.1	18.5	12.5	13.3	12.1	9.6	11.6	25	0	45

**Table 2. Distribution of The HPV Infection (n=578)**

HPV Genotype	Sample numbers	Single Infection (%) n=176	Multiple infections (%) n=81	Double Infections (%) n=53	Triple Infections (%) n=15	Four or more Infections (%) n=13
6	40	22 (12.6)	18 (22.2)	9 (17.0)	4 (26.7)	5 (38.5)
11	34	16 (9.1)	18 (22.2)	9 (17.0)	3 (20.0)	6 (46.2)
42	29	15 (8.6)	14 (17.3)	10 (18.9)	2 (13.3)	2 (15.4)
43	2	1 (0.6)	1 (1.2)	0 (0)	1 (6.7)	0 (0)
44	6	3 (1.7)	3 (3.7)	2 (3.8)	0 (0)	1 (7.7)
16	53	29 (16.5)	24 (29.6)	11 (20.8)	6 (40.0)	7 (53.8)
18	12	3 (1.7)	9 (11.1)	2 (3.8)	2 (13.3)	5 (38.5)
31	18	9 (5.1)	9 (11.1)	4 (7.5)	1 (6.7)	4 (30.8)
33	9	2 (1.1)	7 (8.6)	4 (7.5)	1 (6.7)	2 (15.4)
35	4	3 (1.7)	1 (1.2)	0 (0)	0 (0)	1 (7.7)
39	22	8 (4.5)	14 (17.3)	7 (13.2)	2 (13.3)	5 (38.5)
45	2	0 (0)	2 (2.5)	0 (0)	1 (6.7)	1 (7.7)
51	7	1 (0.6)	6 (7.4)	3 (5.7)	2 (13.3)	1 (7.7)
52	45	25 (14.3)	20 (24.7)	12 (22.6)	4 (26.7)	4 (30.8)
53	20	7 (4.0)	13 (16.0)	9 (17.0)	1 (6.7)	3 (23.1)
56	11	3 (1.7)	8 (9.9)	3 (5.7)	3 (20.0)	2 (15.4)
58	22	9 (5.1)	13 (16.0)	5 (9.4)	1 (6.7)	7 (53.8)
59	15	6 (3.4)	9 (11.1)	4 (7.5)	2 (13.3)	3 (23.1)
66	10	3 (1.7)	7 (8.6)	2 (3.8)	3 (20.0)	2 (15.4)
68	16	4 (2.3)	12 (14.8)	7 (13.2)	2 (13.3)	3 (23.1)
CP8304	15	7 (4.0)	8 (9.9)	2 (3.8)	4 (26.7)	2 (15.4)

17 to 79 years, the median age is 37.49±11.71) attending in No. 150 Central Hospital of PLA, Luoyang, Henan, are included in the present study (Table 1). All cervical samples from females were found to be positive for  $\beta$ -globin, indicating that the extracted DNA was adequate to be analyzed further. Overall, 257 women (44.5%, 257/578) were found to be HPV positive for any HPV DNA, of whom 35.1% (203/578) were found to be HR-HPV infection, substantially higher than the LR-HPV infection (16.8%, 97/578). Among the 257 women with HPV infection, there were two peaks of HPV infection among the women, the first was 71.4% in the  $\leq 20$  year-old group and the second was 65.0% in the  $>60$  year-old groups ( $\chi^2=22.279$ ,  $p<0.05$ ). In different age groups, women infected with the HR-HPV or LR-HPV were significantly different ( $\chi^2=7.019$ ,  $p<0.05$ ). Among the women infected with the HR-HPV, the first peak was within the  $>60$  year-old group (55.0%), and the second peak was within the 51-55 year-old group (50.0%) ( $\chi^2=19.497$ ,  $p<0.05$ ). The prevalence of the LR-HPV exhibited the first peak in the  $\leq 20$  age group, and decreased slowly until the second peak was in the  $>60$  year-old group.

Among women with the HPV infection, HPV 16 was the most prevalent genotype (9.2%, 53/578), followed by HPV 52 (7.8%, 45/578), HPV 6 (6.9%, 40/578), HPV 11 (5.9%, 34/578), HPV 42 (5.0%, 29/578). There are significant differences in the HPV infection among the 21 different HPV genotypes ( $p<0.05$ ).

#### Distribution of the HPV Genotype

The incidence of single and multiple HPV infection is shown in Table 2. Single HPV infection was detected in 30.4% (176/578) of all the involved patients, and the multiple-type infections rate was 14.0% (81/578). Patients infected with more than one type HPV were classified into the multiple-type infections group with the incidence of the double, triple, four or more than four types HPV infections was 9.2% (53/578), 2.6% (15/578), 2.2% (13/578) separately. The rate of the single- and multiple-

type infections in different HPV genotype exhibited significant different ( $p<0.05$ ). In the single group, HPV 16 was the most prevalent (16.5%), followed by HPV 52 (14.3%), HPV 6 (12.6%), HPV 42 (8.6%) and HPV 31 (5.1%). In the multiple-type infections, the most prevalent genotype was HPV 16 (29.6%), followed by HPV 52 (24.7%), HPV 6 (22.2%), HPV 11 (22.2%), HPV 42 (17.3%) and HPV 39 (17.3%).

In the group of double infections, HPV 52 (22.6%), rather than HPV 16 (20.8%), was the most commonly identified type, followed by HPV 42 (18.9%), HPV 6 (17.0%), HPV 11 (17.0%) and HPV 53 (17.0%). In the group of triple infections, HPV 16 (40.0%) was the most frequent genotype, followed by HPV 52 (26.7%), HPV 6 (26.7%) and CP8304 (26.7%). In the group of four or more genotypes infections, HPV 16 and HPV 58 were the most frequent (53.8%), followed by HPV 11 (46.2%), HPV 6 (38.5%), HPV 18 (38.5%), and HPV 39 (38.5%).

#### Discussion

In the present study, the prevalence of HPV infection among the females who underwent the gynecological outpatient clinic in Henan was evaluated by commercially available HPV DNA Chip. A high rate of HPV-DNA infection (44.4%) was observed in specimens taken from patients ranged from 17 to 79 years, and 35.1% was the HR-HPV types. The prevalence of HPV infection among females attending the cervical cancer screening varies in different regions of China, for example, in Shenzhen city, 13.8% of the volunteers was HPV positive; in Shanghai city, southeast of China, the infection rate was 30.2% (Xue et al., 2009; Wang et al., 2013). In the present study, the prevalence of HPV infection showed a significant difference among patients in the age groups ( $\chi^2=22.279$ ,  $p<0.05$ ). The first peak of HPV infection (71.4%) occurred in the  $\leq 20$  years group, might be due to lack of adaptive immune responses and susceptible to the new LR-HPV or HR-HPV infection (Ye et al., 2010).

The second peak (65.0%) was observed in the >60 years group, that may be partly explained by viral persistence or reactivation of latent HPV due to the physiologic and immunologic dysregulation caused by hormone fluctuations at menopausal transition (Althoff et al., 2009; Xue et al., 2009). The highest prevalence of the HR-HPV infection (55.0%) appeared in the >60 years group was more likely for viral persistence than for acquisition of new infection (Castle et al., 2005; Goodman et al., 2008). It was reported that the persistence rate of the HR-HPV infection was higher than LR-HPV infection, and it was a strong predictor for the development of CIN2/3 and invasive cervical cancer (Dalstein et al., 2003; Datta et al., 2012). The previous work found that the HR-HPV DNA was positive among 80% of patients with cervical cancer, so more inspections, including cytology and even colposcopy, should be proceed among women aged >60 years for the prevention of cervical cancer (Wang et al., 2013).

Our findings showed the most prevalent HPV genotype was HPV 16 (9.2%, 53/578), followed by HPV 52 (7.8%, 45/578), HPV 6 (6.9%, 40/578), HPV 11 (5.9%, 34/578), HPV 42 (5.0%, 29/578) and HPV 58 (3.8%, 22/578), which was similar to that of Beijing city (Hou et al., 2012). In other regions of China, for example, in Zhejiang province, a coastal region in southeast China, the most predominant type was HPV 52 (3.1%), followed by HPV 16 (2.5%), HPV 58 (2.1%), HPV 68 (1.0%) and HPV 81 (0.9%) (Ye et al., 2010); in Shenzhen city, the five most commonly found HPV types were HPV 16 (3.47%), HPV 58 (1.68%), HPV 33 (1.38%), HPV 43 (1.36%) and HPV 18 (1.27%) (Wang et al., 2013). The difference may be due to the geographical and biological interplay between HPV types or variants and host immunogenic factors (Xue et al., 2009).

In summary, the rate of single-type HPV infection was detected in 68.5% (176/257), which was much higher than that of the multiple-type HPV infections (31.5%, 81/257). Females infected with multiple-type HPV could be divided into three categories: (i) high and low risk HPV; (ii) high and high risk HPV; (iii) low and low risk HPV. In the group of double infections, the rate of category (i) was 49.1% (26/53), (ii) was 47.2% (25/53), (iii) was 3.8% (2/53). In the triple infections group, the category (i) was 60% (9/15), the rest was (ii). Women infected with multiple-type HPV were at significantly increased risk of both CIN2+ and HSIL+, when compared with single infection (Dickson et al., 2014).

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