

Prevalence of human papillomavirus and the correlation of HPV infection with cervical disease in Weihai, China

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Summary

Objective: This study investigates the human papillomavirus (HPV) infection rate in female genital tracts, as well as the HPV genotype distribution and HPV correlation with cervical disease in Weihai, Shandong Province, China. **Materials and Methods:** A random sample of 9,460 volunteers was simultaneously screened using gene chips and examined by ThinPrep liquid-based cytology test (TCT). Cervical biopsy samples were collected from women with positive HPV-DNA and abnormal TCT for pathological diagnosis. **Results:** The overall HPV prevalence was 6.93% (656 of 9,460). A total of 753 subjects were infected with HPV subtypes (including multiple HPV infections). Of those with infections, 688 were infected with high-risk (HR) types (91.37%), and 65 were infected with low-risk subtypes (8.63%). The single-infection rate was 63.1%. The prevalence rates of HPV in women aged 20 to 39 years and 40 to 59 years were 7.29% and 6.71%, respectively. The most common genotype was HPV16. The HR genotypes were associated with cervical diseases such as atypical squamous cells of undetermined significance (ASCUS) (37.9%), atypical squamous cells high grade (ASC-H) (42.5%), low grade squamous intraepithelial lesion (LSIL) (50%), and high grade squamous intraepithelial lesion HSIL (66.7%). Cervical biopsy results show that the HPV detection rate increased in the following biopsy samples: cervical intraepithelial neoplasia (CIN) I (74.11%), CIN II (84.31%), CIN III (90.32%), and squamous-cell carcinoma (SCC) (100%). **Conclusions:** The HPV infection rate with associated cervical disease in Weihai is equal to those in foreign countries but is lower than the average rate in China. The prevalence of HPV was higher in young people. The most common HPV genotype was 16, followed by 52 and 58. HR HPV is the most probable infection factor for cervical diseases.

Key words: Human papillomavirus (HPV) Genotype; Cervical diseases; Liquid-based cytology.

Introduction

Human papillomavirus (HPV) particles are double-stranded DNA viruses that are widely distributed in humans and animals. The virus does not cause cross-infection between species, and the infection is limited and non-systemic. Through epithelial tropism, HPV infects a person's skin or mucosal epithelial cells and causes the infected site to exhibit benign and malignant lesions. Cervical cancer (CC) is mostly linked to HPV infection [1, 2]. In particular, high-risk (HR) HPV, which has 13 types, has been a major causative factor in the development of cervical intraepithelial neoplasia (CIN) and CC [3, 4]. An important requirement for a diagnostic and therapeutic approach is that the testing and identification of HR HPV types should be highly sensitive and specific. Vaccines are vital to the prevention and treatment of CC [5]. The HPV vaccine is highly specific. However, the epidemiological distribution of HPV genotypes varies in different countries and regions. Thus, the authors investigated HPV infection in women of childbearing age in Weihai, Shandong Province, China and determined the relationship of the infection with cervical lesions.

Materials and Methods

Subjects

Between October 2008 and April 2009, 9,460 volunteers were randomly selected from the urban city of Weihai and the counties of Wendeng, Rushan, and Rongcheng in Shandong Province, China. In each county, women from towns or villages were randomly selected. An HPV virus check was conducted in married women, particularly in terms of their sexual life (except for hysterectomy, cervical surgery, and menstrual period). All women, who were 20 to 60 years of age, agreed to participate in the study. This study was conducted in accordance with the declaration of Helsinki. This study was conducted with approval from the Ethics Committee of Weihai Municipal Hospital. Written informed consent was obtained from all participants.

Questionnaire interview

Questionnaire survey: an epidemiological survey that included demographic and behavioral indicators was conducted. The demographic indicators included age, address, economic income, educational level, menstrual marital status, occupation, and spouse profession. The behavioral informatics indices included age at first sexual experience, number of sexual partners, contraceptive methods, number of pregnancies, mode of delivery, menopause, and smoking. The questionnaire survey included a self-administered questionnaire and an interview.

Specimen collection and gynecological examination

Gynecologists who passed the unified training performed the routine gynecological examination and sample collection and obtained

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Table 1. — Various types of HPV detection times and percentage of patients.

HPV types	Cases	Rates	Clinical phenotype	HPV types phenotype	Cases	Rates	Clinical
HPV6	8	1.06%	low-risk	HPV51	37	4.91%	high-risk
HPV11	1	0.13%	low-risk	HPV52	95	12.61%	high-risk
HPV16	168	22.31%	high-risk	HPV53	13	1.73%	probably HR
HPV18	34	4.52%	high-risk	HPV56	41	5.44%	high-risk
HPV31	37	4.91%	high-risk	HPV58	95	12.61%	high-risk
HPV33	43	5.71%	high-risk	HPV59	30	3.98%	high-risk
HPV35	14	1.86%	high-risk	HPV66	12	1.59%	probably HR
HPV39	17	2.26%	high-risk	HPV68	46	6.11%	probably HR
HPV42	9	1.20%	low-risk	HPV81			
HPV43	8	1.06%	low-risk	(cp8304)	15	1.99%	low-risk
HPV44	11	1.46%	low-risk				
HPV45	19	2.52%	high-risk				

the detailed records of the conditions of the vulva, vagina, cervix, uterus, accessories, and vaginal discharge. At the same time, samples of exfoliated cervical cells were collected using a cervical brush. From the location of the cervical squamous columnar junction, the sampler was rotated for three to five full circles in a clockwise direction, brushed into a three-ml cervical cell preservation solution, and stored at 4°C until use.

HPV determination

The cells were centrifuged for five minutes at a relative centrifugal force of 9,660×g. Afterward, the brush and supernate were removed, and the sediments obtained were extracted via alkali lysis using DNA extraction kits.

HPV genotyping via the Hybrio HPV GenoArray test

HPV genotype detection was conducted using an HPV GenoArray test kit. The kit used both DNA PCR amplification and Hybrio's proprietary flow-through hybridization technique to simultaneously identify 21 HPV genotypes, including 13 HR types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68), two probable HR (PHR) types (53, 66), and five low-risk (LR) types [6, 11, 42, 43, 44, and cp8304(81)]. The assay was performed according to the manufacturer protocol. In brief, PCR was performed on a PCR system apparatus [6] using a reaction volume of 25 µl, which contained one µl of the DNA template, 23.25 µl of a PCR mix, and 0.75 µl of DNA Taq polymerase.

TCT

The liquid-based thin-layer cytology test (TCT) adopted the Bethesda classification system as the cytologic diagnostic criteria, as follows: negative, atypical squamous cells of undetermined significance (ASCUS), atypical squamous cells high grade (ASC-H), atypical glandular cells (AGS), low grade squamous intraepithelial lesion (LSIL), high grade squamous intraepithelial lesion (HSIL), and squamous-cell carcinoma (SCC).

Colposcopic biopsy and histopathology

Women with abnormal TCT results (≥ASCUS) underwent colposcopic examination. The columnar junction and transformation zone were observed to assess the lesion, and a punch biopsy was performed. Pathological examination was divided into inflammation, CIN I, CIN II, CIN III, and invasive cervical carcinoma.

Statistical analysis

After reviewing the questionnaire, data were key-entered twice and analyzed using the SPSS 15.0 software. Statistical analysis consisted of (1) the frequency table, which describes the demographic

Table 2. — Single and multiple infection rates.

Types of infection	Number of cases	Ratio
Single	414	63.1%
Double	157	23.9%
Triple	30	4.6%
Four	10	1.5%
Five	4	0.6%

characteristics and other related indicators based on the mean; and (2) the description of the overall infection rate and different characteristics, as well as different types of HR HPV infection.

Results

Overall HPV infection

A total of 9,460 individuals were included in this study. The number of people from rural areas was 4,130, whereas those from the urban areas was 5,330. Of the total, the number of HPV-positive subjects were 656 and the positive rate was 6.93%. Twenty-one subtypes were detected in 753 cases. HR HPV was detected in 688 subjects (91.37%), whereas the low-risk (LR) type was found in 65 cases (8.63%). The number of HPV-positive cases in the rural population was 289 (infection rate, 7.0%), and that in the urban population was 367 (infection rate, 6.89%). No significant difference was found in the infection rates ($p = 0.86$).

Distribution of different HPV genotypes

HPV16 was the most prevalent type (168 cases, 22.31%), followed by HPV58 (95 cases, 12.61%) and HPV52 (95 cases, 12.61%), which were HR HPVs. Meanwhile, the prevalence rate of LR HPVs did not exceed 2% (Table 1).

Multiple HPV infection rate

Table 2 shows the proportions of HPV single infection and multiple infections. From a total of 656 positive cases, 414 (63.1%) involved single infections, whereas 242

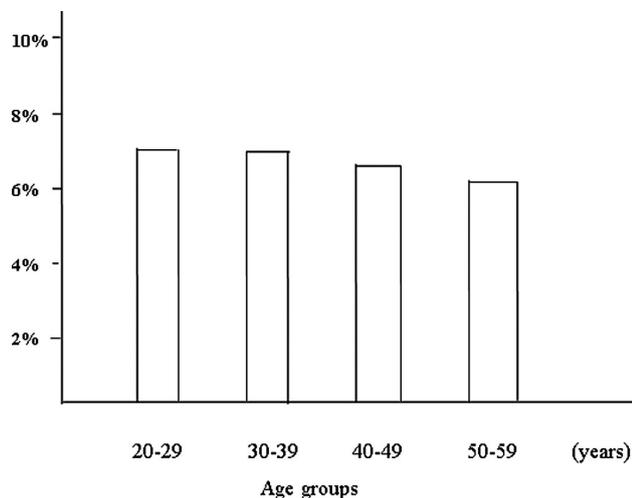


Figure 1. — The prevalence of HPV among women aged 20-29 and 29-39 years was 7.29% and 7.28%, respectively; slightly more than that in 40-49 and 50-59 years which was 6.98% and 6.43%, respectively.

Table 3. — HPV infection and liquid-based cytology relationship

HPV	ASCUS	ASC-H	LSIL	HSIL	AGC	Total
Positive	153 (37.9%)	48 (42.5%)	5 (50%)	10 (66.7%)	2 (9.5%)	218 (38.78%)
Negative	251	65	5	5	19	345
Total	404	113	10	15	21	563

(36.9%) had multiple infections. Double infection was most common type (23.9%) of multiple infections.

HPV infection in different age ranges

The prevalence rates of HPV among women aged 20 to 29 years and 29 to 39 years were 7.29% and 7.28%, respectively. These values were slightly higher than those among the 40 to 49 years and 50 to 59 years age groups (6.98% and 6.43%, respectively) (Figure 1).

HPV infection and liquid-based cytology relationship

The liquid-based cytology results for 563 cases were higher than those of ASCUS. A total of 218 HPV-positive patients were identified (positive rate, 38.7%), whereas 345 patients were HPV-negative (negative rate, 61.3%). The rate of HPV infection increased with the severity of the lesions (Table 3).

TCT \geq ASCUS, HPV subtype distribution

In TCT \geq ASCUS, the total number of HPV-positive detection was 299. Of these, type 16 was the most frequently detected (83 times) and accounted for 27.76%, followed by types 52 (13.71%), 33 (11.04%), and 18 (30 cases, 10.03%) (Table 4).

Table 4. — Liquid-based cytology \geq ASCUS in the distribution of HPV subtypes.

HPV	ASCUS	ASC-H	LSIL	HSIL	AGC	Total
16	60	16	2	5	0	83(27.76%)
18	22	6	0	0	2	30(10.03%)
31	12	0	0	0	0	12(4.01%)
33	31	0	2	0	0	33(11.04%)
35	5	7	2	1	0	15(5.02%)
44	0	6	0	0	0	6(2.00%)
45	0	0	0	1	0	1(0.33%)
51	15	0	0	0	0	15(5.02%)
52	33	7	0	1	0	41(13.71%)
53	5	0	0	0	0	5(1.67%)
56	14	0	0	0	0	14(4.68%)
58	20	0	0	2	0	22(7.36%)
59	8	0	0	0	0	8(2.68%)
68	6	0	0	1	0	7(2.34%)
81	0	7	0	0	0	7(2.34%)
Total	231	49	6	11	2	299

Table 5. — Cervical biopsy and HPV infection.

HPV	CIN I	CIN II	CIN III	SCC	Total
Positive	83 (74.11%)	42 (84.00%)	27 (90.00%)	2 (100%)	154 (79.38%)
Negative	29	8	3	0	40
Total	112	50	30	2	194

$p = 0.16$

Relationship between HPV and biopsy pathology

Women with liquid-based cytology \geq ASCUS underwent electronic colposcopy and biopsy pathology. The number of CIN I patients was 112, of which 83 were HPV-positive (positive rate of 74.11%). The number of CIN II patients was 50, of which 42 were HPV-positive (positive rate of 84.00%). The number of CIN III patients was 31, of which 28 were HPV-positive (positive rate of 90.00%). Meanwhile, two patients were positive for SCC and both were HPV-positive (positive rate of 100%) (Table 5).

Discussion

This study is a large-scale evaluation of the reproductive-tract HPV infection rate and distribution characteristics based on the investigation of married women in Weihai, which is located on the eastern part of Shandong Province in China. HPV is a common sexually transmitted disease pathogen and has an important function in the pathophysiology of CC and cervical diseases. Numerous studies have been conducted on this topic. However, the reported HPV infection rates significantly varied. The present investigation showed that the HPV infection rate among married women in Weihai was 6.93%. This value

was slightly lower than those in Shanxi (14.8%) [7], Shenzhen (11.3%) [8], Shenyang (17%) [9], and Zhejiang (13.3%) [10], but is within the global level (5.0% to 10.0%) [11]. Single infection accounted for 63.1% of all cases and is the most common condition. Meanwhile, the correlation of multiple infections is under investigation. Lee *et al.* [12] concluded that single HPV infections increase the risk of CC by 19.9 times, whereas multiple HPV infections increase the risk by 31.8 times.

Most population screening studies showed a declining prevalence of HPV infection with increasing age [13, 14]. However, the report for Guangdong Province, China is different [12]. The present data showed that the incidence of HPV infection was slightly higher in younger women.

In the present investigation, the most common genotype was HPV-16, followed by HPV-52, HPV-58, HPV-68, HPV-33, HPV-56, HPV-51, HPV-31, HPV-18, and HPV-59. The first five genotypes accounted for 55.67% of the total infections, and all were HR types. This condition was similar to that found in East Guangdong [15, 16]. Based on the analysis of De Sanjosé *et al.* [11], HPV-16 and HPV-18 are prevalent worldwide and account for 32% of all CC causal factors, 87% of cervical diseases, and 75% of invasive CC. HPV-45 and HPV-58 are also related to CC. The investigated sequence in Weihai in terms of decreasing prevalence is HPV-16, HPV-52, and HPV-58, with HPV-18 is the ninth most prevalent subtype. Most statistical evidence in Asia shows that although HPV-16 is the most common genotype, HPV-52 and HPV-58 are also highly prevalent. In Japan, the HPV-52 genotype is the second most prevalent type. In South Korea, HPV-33 and HPV-58 rank second and third in prevalence, respectively [17, 18]. The situation in Weihai is similar to those in Japan and South Korea, mainly because of the close contact between the population of Weihai and those of these two countries.

HR HPV mainly results in squamous intraepithelial lesions and CC. This study demonstrated that the HPV infection rate increased with worsening patient condition (ASCUS 37.9%, ASC-H 42.5%, LSIL 50%, and HSIL 66.7%). HR HPV-16 constituted the largest proportion (27.76%), followed by HPV-52 and HPV-18. These results indicate that the HPV-52 and HPV-18 may also lead to CC or diseases. The cervical biopsy results show that the detection rate of HPV increased in the severe lesions (CIN I, 74.11%; CIN II, 84.00%; CIN III, 90.00%, SCC 100%). Thus, the HR HPV infection rate was positively related to cervical disease deterioration, particularly in high CIN and CC. A number of researchers have shown that CIN I, CIN II, and CIN III exhibit different disease deterioration levels that are associated with the presence of HPV DNA. CIN I is in the free-mode form in benign lesions. The DNA virus does not bind to the host chromosome but simply invades the bottom of squamous cells with low reproduction rate. Meanwhile, CIN III is in the integrated form. In this

disease, the DNA virus integrates and binds with the host chromosome and results in cancer formation and invasion of the bottom of squamous cells. Persistent HR HPV infections during CIN I worsen the condition and can lead to CC. The HPV test is clearly useful in the early diagnosis early treatment of CC as well as in the evaluation of therapeutic effects. This investigation is the first to provide data on HPV infection and HPV genotype distribution in Shandong Province, China. Scientific HPV investigation, HR HPV detection, and research on HPV genotype distribution provide vital data for HPV vaccine research in the present country [19]. This study may contribute to research on CC prevention and can be used in designing effective clinical treatments.

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