Distribution of human papillomavirus genotypes in cervical lesions

LI MA, XIAO CONG, MAI SHI, XIU-HONG WANG, HAI-YAN LIU and MEI-LU BIAN

Department of Gynecology and Obstetrics, China-Japan Friendship Hospital, Beijing 100029, P.R. China

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Abstract. The aims of the present study were to investigate the distribution of human papillomavirus (HPV) genotypes in cervical lesions, and the association between different HPV genotypes and cervical lesions. Between January 2013 and June 2014, the HPV type determinations of nucleic acid by use of fluorescence polymerase chain reaction (PCR) method of 15,192 outpatients in China-Japan Friendship Hospital were performed and the infection status was analyzed. The results showed that: i) 2,366 Cases were HPV positive and 12,826 cases were HPV negative, the overall infection rate was 15.57% (2,366/15,192), in which a single genotype of HPV infection rate was 11.63% (1,767/15,192), and multiple genotypes of HPV infection rate was 3.94% (599/15,192); ii) HPV16, HPV52 and HPV58 infections were the most common HPV genotypes, the infection rates were 3.95% (600/15,192), 2.86% (435/15,192) and 2.67% (406/15,192), respectively; and iii) According to the gold standard of histopathological analysis via hematoxylin-eosin staining, HPV16, HPV52 and HPV58 accounted for 58.80% (154/267) of all CIN2 or above squamous epithelial lesions. Furthermore, three cases with pathological changes of the cervical severe glandular epithelium were all HPV18 infection. The difference was statistically significant (χ^2=60.74, P<0.001). Single HPV subtype infection was primarily associated with HPV16, HPV52 and HPV58. In conclusion, HPV type detection had a may be important in screening of cervical lesions as a difference in pathogenic ability was noted among different HPV genotypes. As cervical cancer is an infectious disease, HPV testing may help detect more precancerous lesions, thus reducing the morbidity and mortality of cervical cancer. HPV16, HPV52 and HPV58 were associated with severe cervical squamous epithelial lesions; HPV18 was associated with cervical severe glandular cell pathological changes, although it was not the most common HPV genotype in China. When positive, a clinical cervical examination should be conducted, including colposcopy and biopsy.

Correspondence to: Dr Mei-Lu Bian, Department of Gynecology and Obstetrics, China-Japan Friendship Hospital, 2 Yinghua Dongjie, Beijing 100029, P.R. China
E-mail: bianmeilu@hotmail.com

Key words: human papilloma virus, genotype, cervical intraepithelial neoplasia, cervical glandular intraepithelial neoplasia

Introduction

Cervical cancer is a type of malignant tumor which was the fourth most common cancer amongst women worldwide in 2012, with 528,000 new cases (1,2). Human papillomavirus (HPV) has been considered to be a major cause of cervical cancer and cervical intraepithelial neoplasia, and high-risk (HR) HPV persistent infection significantly correlates with cervical cancer (3-5). HPV genotyping is being increasingly studied, along with the study of the association between various HPV types and cervical cancer. The importance of its clinical application was proposed by the European Research Organization on Genital Infection and Neoplasia (EUROGIN) in 2010 (6). In 2011, the application of HPV genotyping to screening was proposed by the same organization, which can detect more CIN2-3 patients compared with a cytology test (7). Furthermore, Qiao *et al* (8), Arbyn *et al* (9) and Liu *et al* (10) have proposed that high-risk HPV detection may be used for cervical carcinoma screening in China. Significant reform was made in cervical cancer screening in 2015 after the American Society for Colposcopy and Cervical Pathology proposed that HPV genotyping be used as a cervical cancer screening method (11). The possibility of selecting HPV genotyping as the first choice for cervical carcinoma screening was investigated in the present study based on HPV genotyping data collected in the China-Japan Friendship Hospital (Beijing, China).

Materials and methods

Ethical approval and patient consent. Written informed consent was obtained from all the patients, and this study was approved by the Ethics Committee of the China-Japan Friendship Hospital (Beijing, China).

Study population. A total of 15,192 female outpatients at the China-Japanese Friendship Hospital volunteered to undergo a High-Risk HPV genotyping assay [quantitative polymerase chain reaction (qPCR) method] between January 2013 and June 2014. The average patient age was 32.52±7.77 years. Participants were sexually active and did not receive surgery for uterine diseases. Participants were recruited while in hospital to undergo physical examination, as well as patients that experienced abnormal leucorrhea, vaginal bleeding during sexual intercourse and abnormal vaginal bleeding.

High-risk HPV genotyping assay (qPCR). Exfoliated cells were collected by swab at cervical foci, placed in sterile glass

tube and sealed. Genotyping was performed using a Uterine Cervix Cancer of High-risk HPV Genotype Related Real Time PCR Kit (Liferiver Bio-Tech Corp., San Diego, CA, USA), which is able to detect and distinguish 13 HPV genotypes, including HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68. A total of 13 pairs of genotype-specific primers and 13 Taqman probes (Liferiver Bio-Tech Corp.) were used for the detection and genotyping of the genomic DNA of 13 HPV genotypes using qPCR.

In brief, template DNA was prepared from cervical swab samples taken from 15,192 female outpatients using Nucleic Acid Extraction Reagent, which is a component of High-risk HPV Genotype Related Real Time PCR Kit. Primers were provided in the kit and the total reaction volume was 40 μl. Thermal cycling was performed according to the manufacturer's instructions: 94˚C for 2 min, followed by 40 cycles of 93˚C for 10 sec and 62˚C for 31 sec. PCR amplification and hybridization were performed using the Roche LightCycler (Roche Diagnostics, Basel, Switzerland). Positive and negative controls were performed, as provided by in the kit.

Fluorescent signals of cycle 6-15 were used for baseline setting, and threshold line was set just over the highest point of amplification curve of blank, in which H_2O was used as template. Cycle threshold (Ct) value was used as a marker of virus load, Ct value represents the threshold cycle number of qPCR; higher HPV copy number implies more severe HPV infection in patient and higher risk of developing into cervical cancer. If Ct value is <38 and typical amplification curve is shown, a positive result may be reported; one or more HPV genotypes can be detected, which represent simple and multiple infection respectively. HPV virus load can be divided into seven levels, 10^2-10^7 and >10^7 based on Ct values: When Ct value is <20.0, HPV viral load is >10^7 copies/10^4 cells; Ct value is 20.0-23.3, HPV viral load is 10^7 copies/10^4 cells; Ct value is 23.4-26.7, HPV viral load is 10^6 copies/10^4 cells; Ct value is 26.8-30.1, HPV viral load is 10^5 copies/10^4 cells; Ct value is 30.2-33.5, HPV viral load is 10^4 copies/10^4 cells; Ct value is 33.6-36.9, HPV viral load is 10^3 copies/10^4 cells; and Ct value is 37.0-40.0, HPV viral load is 10^2 copies/10^4 cells. Ct value formula of HPV-positive sample: $Ct = Ct_{Sample} - Ct_{IC} + 28$. If the Ct value of 'Channel CY5' in mixture I [which is able to detect HPV16, 56, 31 and internal control (IC)] is <32, which represents IC, and 'Undetermined' or 'No Ct' is shown in 'Column Ct' of all channels except IC, the result was considered to be negative. If the Ct value was between 38.0 and 40.0, the sample was tested repeatedly; if it remained in that interval and a typical S-shaped amplification curve was shown, a result was considered to be positive. If no typical S-shaped curve is observed, a negative result can be reported.

Liquid-based cytology test (LCT). Slides were produced using AutoCyte Prep (TriPath Imaging, Inc., Becton Dickinson, Burlington, NC, USA), and cytology diagnosis was made based on The 2001 Bethesda system (TBS2001) classification system by American Academy of Family Physicians (12). Cytological results were considered to be positive if the clinical significance is over the grade of ASC-H (atypical squamous cells-cannot exclude high-grade squamous intraepithelial lesion). All LCT tests were reviewed and diagnosed by two cytopathologists independently.

Histopathology. Histopathological diagnosis is the gold standard for the diagnosis of cervical lesions. Cervical biopsy samples were extracted from 648 cases in 2,366 HPV-positive patients, including HPV-positive and LCT-positive patients, as well as LCT negative but HPV16-positive patients, in this study by colposcopy. Biopsy tissue was fixed with 10% formaldehyde, dehydrated, embedded in paraffin and cut into sections (3-4 μm) using a microtome. The sections were subjected to hematoxylin-eosin staining (Beyotime Institute of Biotechnology, Inc., Shanghai, China). Sections were observed under a microscope (BX51; Olympus Corp., Tokyo, Japan), reviewed and independently diagnosed by two pathologists. The histopathological diagnosis standard adopted was the World Health Organization classification of tumors pathology (13). Histopathology grades over CIN2 (including CIN2) were considered to be positive, and histopathology grades below CIN1 (including CIN1) were considered to be negative.

Statistical analysis. SPSS 17.0 statistical software (SPSS, Inc., Chicago, IL, USA) was applied for analysis, disaggregated data describing the percentage, rate comparisons using χ^2 test, numerical data (mean ± standard deviation) describing both groups were compared according to homogeneity of variance, nonparametric tests (Mann-Whitney u-test). $P<0.05$ was considered to indicate a statistically significant difference.

Results

Age distribution of participants. The HPV infection rate was found to be 15.57% (2,366/15,192) in 15,192 female participants, with an average age of 32.52±7.77 years. The average age of the 2,366 HPV-positive patients was 34.53±9.62 years, and was 32.15±7.32 years for the 12,826 HPV-negative patients. There was a significant difference ($Z=-9.815$, $P<0.001$) between the ages of the HPV-positive and negative participants. Single genotype infection number was 1,767 in HPV-positive patients and the infection rate was 11.63% (1,767/15,192). The multiple infection number was 599 and the infection rate was 3.94% (599/15,192).

Results of high risk HPV genotyping assay (qPCR). A total of 13 HPV genotypes were detected in this study: HPV16 (3.95%, 600/15,192), HPV52 (2.86%, 435/15,192), HPV58 (2.67%, 406/15,192), HPV39 (1.98%, 301/15,192), HPV68 (1.72%, 262/15,192), HPV51 (1.49%, 226/15,192), HPV56 (1.38%, 209/15,192), HPV59 (1.36%, 207/15,192), HPV18 (1.01%, 153/15,192), HPV31 (0.68%, 104/15,192), HPV33 (0.68%, 104/15,192), HPV35 (0.64%, 97/15,192) and HPV45 (0.31%, 47/15,192).

Among different age groups, the HPV infection rate varied. From 25 to 29 years old, 838 cases were HPV positive and 5,434 cases were HPV negative, the infection rate was 13.36% (838/6,272). From 30 to 39 years old, 947 cases were HPV positive and 5,571 cases were HPV negative, the infection rate was 14.53% (947/6,518), from 40 to 49 years old, 343 cases were HPV positive and 1,313 cases were HPV negative, the infection rate was 20.71% (343/1,656), from 50 to 59 years old, 187 cases were HPV positive and 431 cases were HPV negative, the infection rate was 30.26% (187/618),

Table I. Association between HPV type and histopathological diagnosis.

Diagnosis	HPV16	HPV18	HPV31	HPV33	HPV35	HPV39	HPV45	HPV51	HPV52	HPV56	HPV58	HPV59	HPV68	Total
Normal	33	25	7	14	14	46	8	43	59	37	55	17	21	379
CIN1	34	7	11	6	7	23	5	21	24	27	31	8	15	219
CGIN1	-	-	1	-	-	1	-	-	2	4	-	-	1	9
CIN2	16	2	6	5	4	8	1	6	13	8	15	6	3	93
CIN3	57	6	6	10	5	5	2	3	24	13	20	4	3	158
SC	6	2	1	-	1	1	-	1	-	1	3	-	-	16
CGIN3	-	1	-	-	-	-	-	-	-	-	-	-	-	1
AC	-	2	-	-	-	-	-	-	-	-	-	-	-	2
EC	-	-	-	-	-	-	-	1	1	-	-	-	-	2
Total	146	45	32	35	31	84	16	75	123	90	124	35	43	879

HPV, human papilloma virus; CIN, cervical intraepithelial neoplasia; SC, squamous cell carcinoma; CGIN, cervical glandular intraepithelial neoplasia; AC, adenocarcinoma; EC, endometrial carcinoma.

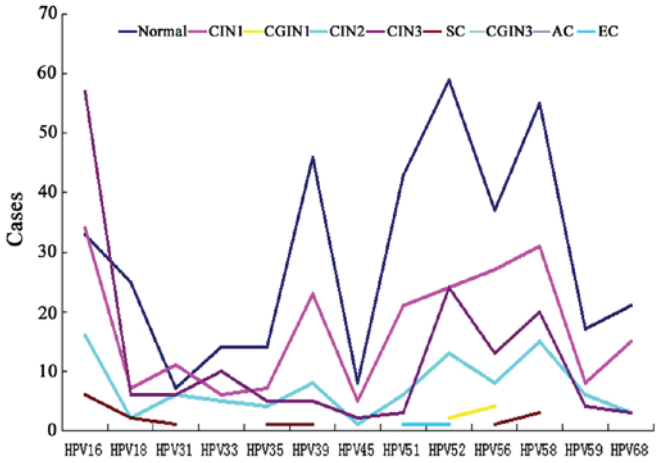

Figure 1. Association between HPV types and histopathological results. CIN, cervical intraepithelial neoplasia; CGIN, cervical glandular intraepithelial neoplasia; SC, squamous cell carcinoma; AC, adenocarcinoma; EC, endometrial carcinoma; HPV, human papillomavirus.

and >60 years old, 51 cases were HPV positive and 77 cases were HPV negative, the infection rate was 39.84% (51/128).

There was a significant difference in HPV infection rate among all age groups (χ^2=220.729, P<0.001). Infection rates increased between young and older women, peaking in the group aged >60 years old.

Age distributions were different between single and multiple infection patients. From 25 to 29 years old, 838 cases exhibited a single infection. From 30 to 39 years old, 929 cases were single infection and 18 cases were multiple infection. From 40 to 49 years old, 343 cases were multiple infection. From 50 to 59 years old, 187 cases were multiple infection and at >60 years old, 51 cases were multiple infection.

Age distributions were significantly different between single and multiple infection patients (χ^2=2,272.609, P<0.001).

Histopathological results. Among the 648 patients that underwent histopathological diagnosis in this study, there were 15 cases with squamous cell carcinoma, 116 cases with CIN3, 66 cases with CIN2, 151 cases with CIN1, 12 cases with gland cell lesion (including 2 cases with cervical gland cancer, 1 case with endomertrial adenocarcinoma, 1 case with CGIN3 and 8 cases with CGIN1) and 288 histopathologically negative but HPV-positive cases; the ratio of patients over CIN2 stage was 31.02% (201/648).

If each HPV genotype had been counted respectively in multiple infection patients, and gland cell lesion accompanied by CIN had been counted respectively, as previously described (14), the total case number would have been 879, including 16 cases with squamous cell carcinoma, 158 cases with CIN3, 93 cases with CIN2, 219 cases with CIN1, 14 cases with gland cell lesion, and 379 histopathologically negative but HPV-positive cases (Table I and Fig. 1).

Cervical squamous epithelial lesions over CIN2 were associated with HPV16, HPV52 and HPV58, which accounted for 58.8% (154/267) of all cases with cervical squamous epithelial lesions over CIN2: i) 38 Cases with diagnosis of over CIN2 in all 461 cases that were LCT-negative but HPV-positive that provided histopathological samples; ii) 8 cases with

Table II. Association between single or multiple HPV infections and cervical lesions.

Histopathological diagnosis	HPV16		HPV18		HPV31		HPV33		HPV35		HPV39		HPV45		HPV51		HPV52		HPV56		HPV58		HPV59		HPV68		Total
	S	M	S	M	S	M	S	M	S	M	S	M	S	M	S	M	S	M	S	M	S	M	S	M	S	M	
Normal	18	15	10	15	4	3	8	6	6	8	20	26	6	2	32	11	37	22	19	18	35	20	12	5	6	15	379
CIN1	21	13	5	2	7	4	5	1	2	5	5	18	1	4	11	10	13	11	17	10	14	17	3	5	1	14	219
CGIN1	-	-	-	-	1	-	-	-	-	-	-	1	-	-	-	-	2	-	4	-	-	-	-	-	-	1	9
CIN2	8	8	1	1	4	2	5	-	1	3	2	6	-	1	3	3	9	4	1	7	6	9	4	2	1	2	93
CIN3	45	12	2	4	3	3	4	6	3	2	-	5	-	2	2	1	12	12	7	6	10	10	3	1	-	3	158
SC	5	1	2	-	1	-	-	-	1	-	1	-	-	-	-	1	-	-	1	-	3	-	-	-	-	-	16
CGIN3	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
AC	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
EC	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1	-	-	-	-	-	-	-	-	2
Total	97	49	23	22	20	12	22	13	13	18	28	56	7	9	48	27	73	50	49	41	68	56	22	13	8	35	879

HPV, human papilloma virus; CIN, cervical intraepithelial neoplasia; SC, squamous cell carcinoma; CGIN, cervical glandular intraepithelial neoplasia; AC, adenocarcinoma; EC, endometrial carcinoma.

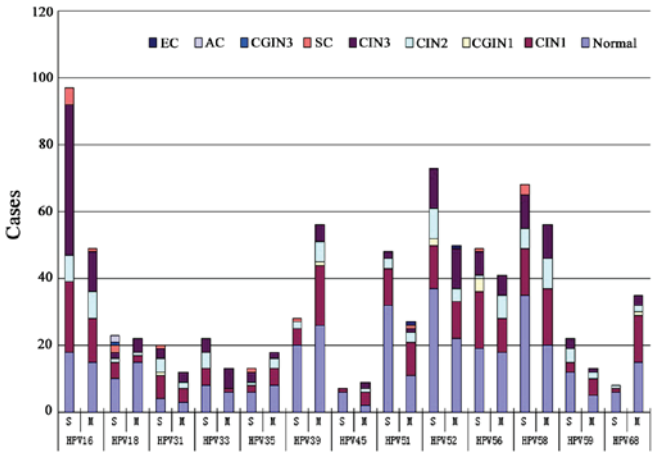

Figure 2. Association between HPV infection and histopathological results. EC, endometrial carcinoma; AC, adenocarcinoma; CGIN, cervical glandular intraepithelial neoplasia; SC, squamous cell carcinoma; CIN, cervical intraepithelial neoplasia; HPV, human papillomavirus.

diagnosis of over CIN2 in 46 cases that were LCT-negative but HPV16-positive; iii) 30 cases with diagnosis of CIN2 or higher in 415 cases that were LCT-negative and positive for other HPV genotypes (with the exception of HPV16); and iv) 23 cases with diagnosis of over CIN2 in 171 cases of 25-30 year old women. The leading cause of severe glandular epithelial lesions of the cervix was HPV18. A total of 3 cases in this study were HPV18 infection (100%, 3/3), of which one case of endometrial adenocarcinoma is HPV51 and HPV52 multiple infection. There was only one case that exhibited endometrial adenocarcinoma with HPV51 and HPV52 co-infection, further studies are required to confirm the association between this lesion classification and these two HPV types (χ^2=60.74, P<0.001).

Association between single or multiple HPV infections and cervical lesions in which the single HPV subtype infection was the primary mode for HPV16, HPV52 and HPV58. The ratio of HPV16 infection caused by squamous epithelial lesions (CIN2 or above) was highest, which accounted for 21.72% (58/267), and was higher compared with the ratio of HPV52, the second highest genotype, which accounted for 7.87% (21/267). The high grade lesions of cervical gland epithelial tissue were associated with HPV18 single infection, accounting for 100% (3/3). HPV39 and HPV68 frequently appeared with other HPV genotypes, which were primarily associated with CIN1 or minor squamous lesions, in which HPV39 related CIN1 or minor accounted for 80% (44/55) and HPV68 accounted for 85.29% (29/34) (Table II and Fig. 2).

Association between HPV viral load and cervical lesions. Viral load values for different HPV genotypes are shown in Table III and Fig. 3. The viral loads of the HPV16, HPV52 and HPV58 genotypes were primarily medium (10^4-10^6 viral load) or high (≥10^7 viral load), whereas HPV39 and HPV68 were low (≤10^3 viral load). The HPV viral load of 40 cases was 10^2 copies/10^4 cells, of which 8 cases were of a histopathology grade ≥CIN2, and 32 cases were ≤CIN1. A total of 97 cases had 10^3 copies/10^4 cells, of which 17 cases were ≥CIN2, and 80 cases were ≤CIN1. A total of 133 cases were 10^4 copies/10^4 cells, of

Table III. Viral load of different HPV types.

HPV copies (/10^4cells)	HPV16	HPV18	HPV31	HPV33	HPV35	HPV39	HPV45	HPV51	HPV52	HPV56	HPV58	HPV59	HPV68	Total
10^2	42	26	5	13	16	71	10	51	92	28	59	48	99	560
10^3	68	31	19	16	23	71	9	33	97	29	37	32	65	530
10^4	86	32	18	20	20	49	10	35	95	34	57	35	54	545
10^5	148	25	20	26	17	36	11	42	92	32	81	33	27	590
10^6	134	21	22	19	11	30	6	34	43	26	81	25	13	465
10^7	88	16	12	10	6	28	1	22	10	33	59	23	2	310
$>10^7$	34	2	8	0	4	16	0	9	6	27	32	11	2	151
Total	600	153	104	104	97	301	47	226	435	209	406	207	262	3,151

HPV, human papillomavirus.

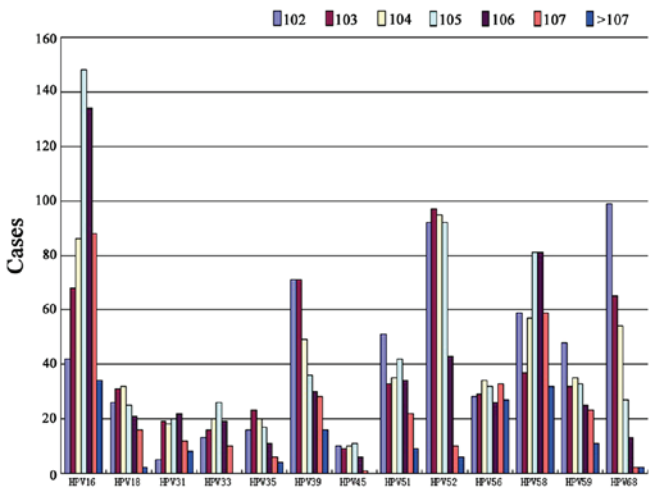

Figure 3. Viral load of different HPV types. (HPV, human papilloma virus).

which 34 cases were ≥CIN2, and 99 cases were ≤CIN1. A total of 163 cases were 10^5 copies/10^4 cells, of which 43 cases were ≥CIN2, and 120 cases were ≤CIN1. A total of 172 cases were 10^6 copies/10^4 cells, of which 73 cases were ≥CIN2 and 99 cases were ≤CIN1. A total of 144 cases were 10^7 copies/10^4 cells, of which 50 cases were ≥CIN2, and 94 cases were ≤CIN1. A total of 130 cases were $>10^7$ copies/10^4 cells, of which 47 cases were ≥CIN2 and 83 cases were ≤CIN1.

The percentages of the viral loads were as follows: 4.55% (40/879) for $10^2/10^4$ cells; 11.04% (97/879) for $10^3/10^4$ cells; 15.13% (133/879) for $10^4/10^4$ cells; 18.54% (163/879) for $10^5/10^4$ cells; 19.57% (172/879) for $10^6/10^4$ cells; 16.38% (144/879) for $10^7/10^4$ cells; and 14.79% (130/879) for $>10^7/10^4$ cells.

The viral loads of squamous cell carcinoma, adenocarcinoma and endometrial carcinoma patients were $>10^4/10^4$ cells in all patients (16/16 for squamous cell carcinoma, 2/2 for adenocarcinoma and 2/2 for endometrial carcinoma). The ratio of the viral load of $>10^4/10^4$ cells accounted for 87.10% (81/93) in CIN2 and 91.77% (145/158) in CIN3, which suggested that the cervical lesion type was associated with viral load. The percentage of cervical lesions of CIN2 or above was 18.25% (25/137) in patients with viral load of $\leq 10^3/10^4$ cells and 33.29% (247/742) for $>10^4/10^4$ cells viral load patients. There was a significant difference (χ^2=27.06, P<0.001) between these two groups.

Discussion

The incidence and mortality rates of cervical carcinoma in China accounted for ~1/3 of the global total in 2002 (15). Early prevention, early detection and early treatment are the key to reduce morbidity and mortality. The development of cervical carcinoma is a gradual process, and may require ≥10 years to develop into invasive cervical carcinoma from dysplasia (16). Persistent HPV infection is the most important risk factor for cervical carcinoma, and high-risk HPV and cervical carcinoma are closely correlated (17,18).

According to a worldwide retrospective cross-sectional study conducted in 2010, the top 8 invasive cervical carcinoma HPV genotypes are HPV16, HPV18, HPV31, HPV33, HPV35,

HPV45, HPV52 and HPV58 in Europe, America, Africa, Asia and Pacific areas (3). The overall HPV infection rate was 15.57% (2,366/15,192) in the present study, and the most common genotypes are HPV16 (3.95%, 600/15,192), HPV52 (2.86% 435/15,192) and HPV58 (2.67% 406/15,192). In previous studies by Chen *et al* (19), Shi *et al* (20) and Li *et al* (21), the HPV infection rates were 19.7% in Xinjiang (China), 32% in Changsha (China) and 9.9% in Beijing (China), respectively, and the most common genotypes were HPV16/HPV58/HPV18, HPV52/HPV16/HPV58 and HPV16/HPV58/HPV33, respectively.

It remains to be further verified by considerably large sample numbers whether cervical carcinoma-related HPV genotype prevalence in China is different from that in the rest of the world.

Previous studies (22,23) have demonstrated that there was a difference in pathogenic ability among different HPV genotypes, and that infection with high-risk HPV genotypes in particular may pose a health risk to female patients. High-risk HPV genotype infections were the risk factors of the lesions diagnosed CIN 2 or higher. When HPV16/18-positive, colposcopy should be performed regardless of the LCT results. In 2007, the International Agency for Research on Cancer proposed that the HPV test may be used as a primary screening method for cervical carcinoma (22). In 2012, the guidelines of the American Congress of Obstetricians and Gynecologists and the American Society for Colposcopy and Cervical Pathology stated that women infected with HPV16 and HPV18 should be examined by colposcopy, even if cytologically negative (23). In the present study, there were 21.05% (8/38) of HPV16-positive cases in patients with LCT negative results and CIN2 or above in histopathologic diagnosis. Therefore, direct HPV genotyping should be considered, if the required laboratory facilities are available. For example, the Cobas HPV genotyping assay (Roche Diagnostics, Risch-Rotkreuz, Switzerland), which was approved by the American Food and Drug Administration in April 2014, may be used as a method for cervical disease screening. Recent updates to cervical lesion screening guidelines indicate the increasing importance of HPV genotyping (22,24,25).

In a previous study, HPV16 and HPV18 were considered as the most two common cervical carcinoma related high-risk genotypes (26). In the present study, cervical lesions of CIN2 or above accounted for 31.02% (201/648) of 648 HPV positive cases with histopathological data. The infection rate of HPV16 was the highest, 29.59% (79/267) in patients with cervical squamous intraepithelial lesions of CIN2 or above and higher compared with that of HPV58, the second highest genotype accounting for 14.23% (38/267). HPV18 infection rate was 5.12% (45/879) overall, but 3.75% (10/267) in patients with cervical squamous intraepithelial lesions of CIN2 or above. However, HPV18 was important in severe cervical glandular epithelial lesions, 1 case of CGIN3 and 2 cases of cervical adenocarcinoma were all associated with HPV18 single infection in the present study. Cervical glandular epithelial lesions are easily neglected, as their locations are frequently too deep to detect and there have been few studies investigating cervical glandular epithelial lesions. Cervical canal should be examined to reduce misdiagnosis of cervical glandular epithelial lesions, if HPV18 infection is detected. In addition, higher viral load indicates higher possibility of severe cervical lesions. In the present study, the incidence of severe cervical lesions in the group with the viral load $>10^4$ was higher compared with the $<10^4$ group.

There are three strategies of cervical carcinoma screening for healthy women in the USA (22,24,25,27): i) Cytological screening alone for women over 21-year-old; ii) HPV screening was the prime choice for women >25-year-old; and iii) cytological and HPV joint screening for women >30-year-old. The screening strategies above are for healthy women, but this study is opportunistic screening. In the present study, participants between 25 and 30-years-old with cervical lesions of CIN2 or above accounted for 0.37% (23/6,272) in that age group, whereas the incidence was 2.00% (178/8,920) in the group >30-years-old. According to the results above, the incidence still existed in the group of 25-30-year-old women, although the incidence was low. Therefore, young women should meet at least one of following requirements before selecting HPV genotyping as the first screen method: i) 25-30 Years old with previous abnormal LCT history; and ii) good financial conditions. In general, HPV genotyping of cervical carcinoma screening is first choice for women >30-years-old.

In conclusion, HPV genotyping indicates that patients with HPV16, HPV52 and HPV58 infections may be particularly susceptible to high-grade cervical intraepithelial lesions, and the endocervix should be examined for patients with HPV18 infection. Thereby, the detection rate of severe cervical squamous intraepithelial lesions and severe glandular intraepithelial lesions may be improved and the incidence of cervical carcinoma may be reduced. HPV genotyping is feasible and economical as the first choice of opportunistic screening in tertiary hospitals.

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