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Distribution of HPV genotypes and the relationship with CIN

Hong Ma[1], Yumei Li[1], Zhiqiang Chen[1], Liping Liu[1], Hui Xu[1], Xiaoyue Yang[2]

[1]Dermatology and Venereology Department, [2]Gynaecology and Obstetrics Department, Jiangsu University Hospital, Zhenjiang City (China)

Summary

Objective: To study the distribution of human papillomavirus (HPV) genotypes in population of hospital opportunistic screening and the relationship with cervical intraepithelial neoplasia (CIN) among women. *Materials and Methods:* A retrospective analysis was performed in 14,991 women who were undergoing HPV genotype testing and 253 inpatients who were undergoing conization pathological with CIN and with pre-HPV test at Jiangsu University Hospital between July 2013 and July 2015. *Results:* Of these, 25.99% were infected by HPV and 20.37% for high-risk HPV (hr-HPV), the highest prevalence of hr-HPV and multiple hr-HPV were all in the 55- to 64-year-old group ($\chi^2 = 37.125$, $p < 0.01$, $\chi^2 = 43.668$, $p < 0.01$). The most prevalent genotype is HPV52, then descending order were HPV16 and 58. On the basis of 253 inpatients, CIN I were 78, CIN II were 67, CIN III were 108. Before conization, hr-HPV positive in CIN I lesions were 74.36% (58/78) and CIN II were 94.03% (63/67), while in CIN III lesions there were 94.44% (102/108) (χ^2=17.936, $p < 0.01$). The most frequent HPV genotype was HPV 16, followed by HPV 58 and 52. Infection with multiple- hr-HPV seemed to have no relationship with the severity of pathology increased (χ^2=1.888, $p > 0.01$). *Conclusion:* This study established the prevalence of HPV genotypes that is similar to the population of CIN, except HPV 16 and HPV 52, 58 were also found in a large proportion of women, HPV 18 was not the most important. Therefore, in the future, a HPV vaccine covering HPV 52/16/58 should be used in this region compared to the currently available vaccine for the prevention strategy in the population

Key words: Human papillomavirus (HPV); Genotype; Cervical intraepithelial neoplasia (CIN).

Introduction

Cervical cancer (CC) is the fourth most common cancer in women, and the seventh overall, with an estimated 528,000 new cases and 266,000 deaths in 2012, and a large majority (around 85%) deaths occurs in the less developed countries [1], that is more likely to be due to the lack of screening and effective prevention. And according to World Health Organization statistical data, about 75,000 new cervical cancer cases were estimated each year, while 33,000 deaths in 2008 in China [2]. Cervical intraepithelial neoplasia (CIN) is well-known precancerous lesions of cervical cancer [3, 4]; if women are with untreated high grade CIN, they are at high risk of CC [5].

As we all known, human papillomavirus (HPV) is the most common sexually transmitted infections among women in worldwide, more than 80% of the population infected at some time in their life. However the persistent high risk HPV (hr-HPV) genotypes infections was one of the causes of majority CCs and its precursor lesions [6]. Therefore, the knowledge of the regional HPV genotype is crucial to guiding estimates of the effectiveness of vaccination programs, health economic calculations, and the development of improved vaccines to reduce the incidence and mortality of cervical lesions.

The present study aims to evaluate the prevalence of HPV infection in population of hospital opportunistic screening in the North of Yangtze River Delta women and in patients with cervical cytological abnormalities.

Materials and Methods

The authors retrospectively reviewed the records of 14,991 people who attended the Cervical Disease Diagnosis and Treatment Center and Dermato-venerological Department, and 253 in-patients with histologically confirmed CIN I-III, who had been treated by a loop electrical excision procedure (LEEP) and both pre-LEEP HPV test at the Department of Gynecology of Jiangsu University Hospital between July 2013 and July 2015. This study did not disclose the patients' specific information in order to protect their privacy.This study protocol was approved by ethics committee of Jiangsu University.

The cervical samples were obtained via cytobrush from each woman. An HPV test kit was used to perform HPV genotyping. It was used in combining DNA amplification and DNA reverse hybridization technique. HPV Blot contains 23 types of genotypes, including four low-risk types (6, 11, 42, 43), 17 high-risk types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 73, 82, 83), and two unknown-risk types (81 and 53).

Cervical excisional specimens (LEEP) were formalin fixed, paraffin embedded, and cut into 4-mm thin sections. Adjacent histologic sections of each sample were read for endpoint determination by a panel of two pathologists.

The data were processed using SPSS 19.0 software package. The Chi-square test (χ^2) was used to compare HPV prevalence across groups. Statistical significance was determined at $p < 0.05$ level.

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Table 1. — *The prevalence of HPV and hr-HPV infection according to age.*

Age	Total numbers	HPV numbers	Prevalence of HPV (%)	hr-HPV numbers	Prevalence of hr-HPV (%)
< 25	702	216	30.77	169	24.07
25-34	4736	1210	25.55	957	20.21
35-44	5167	1253	24.25	978	18.93
45-54	3239	872	26.92	677	20.9
55-64	862	277	32.13	215	24.94
> 64	285	69	24.21	58	20.35
Total	14991	3897	25.99	3054	20.37
χ^2		35.793		37.125	
p		< 0.01		< 0.01	

Comparison between age groups:the prevalances of HPV and hr-HPV in 55-64 and <25 age groups are higher than other groups, P<0.01 HPV:human papillomavirus
hr-HPV:high risk human papillomavirus

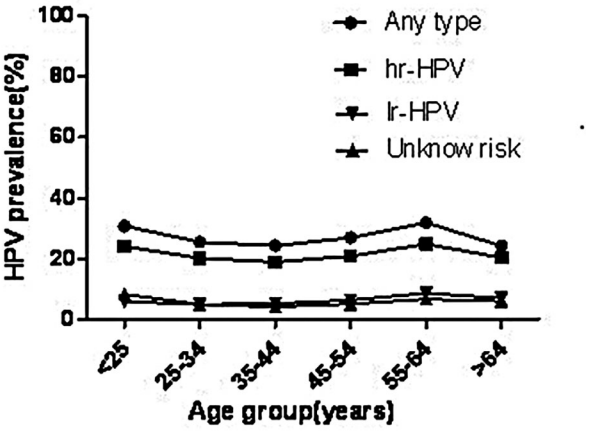

Figure 1. — HPV prevalence according to age. Either hr-HPV or Ir-HPV, even unknown HPV, the prevalence of HPV and hr-HPV was highest in 55-64 and < 25 age groups.
hr-HPV: high risk human papillomavirus; Ir-HPV: low risk human papillomavirus.

Results

A total of 14,991 cervical samples were analyzed between the age of 18 and 80 years from the outpatient of Jiangsu University Hospital from July 2013 to July 2015 (average age 39.33 ± 10.72 years), excluding rechecked cases. The age group among the women enrolled in the study is reported in Table 1. HPV was detected in 3,897 samples and the overall prevalence was 25.99%

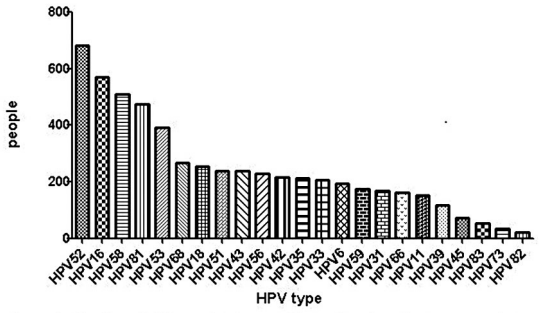

Figure 2. — Distribution of different HPV genotypes. The DNA Chip includes 17 hr-HPVs (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 73, 82, and 83), low-risk HPVs (6, 11, 42, and 43), and unknown HPV types (81 and 53) among the population.

Table 2. — *Distribution of hr-HPV, in single or multiple infections, according to age.*

Age	Total numbers	Single infections (n, %)		Multiple infections (n, %)	
< 25	702	132	18.8	84	11.97
25-34	4736	868	18.33	342	7.22
35-44	5167	899	17.4	354	6.85
45-54	3239	606	18.71	266	8.21
55-64	862	173	20.07	104	12.06
> 64	285	38	13.33	31	10.88
Total	14991	2716	18.12	1181	7.88
χ^2		9.539		51.338	
p		0.089		<0.01	
Age	Total numbers	Single hr-HPV infections (n, %)		Multiple hr-HPV infections (n, %)	
< 25	702	115	16.38	54	7.69
25-34	4736	752	15.87	205	4.33
35-44	5167	772	14.94	206	3.99
45-54	3239	532	16.42	145	4.48
55-64	862	148	17.17	67	7.77
> 64	285	38	13.33	20	7.01
Total	14991	2357	15.72	697	4.67
$\chi 2$		6.494		43.668	
p		0.261		< 0.01	

Comparison between age groups: the prevalances of multiple HPV and hr-HPV in 55-64 and < 25 age groups are higher than other groups (p < 0.01). The prevalances of single HPV and hr-HPV in all groups show no differences (p > 0.05). HPV: human papillomavirus hr-HPV: high risk human papillomavirus.

Table 3. — *Distribution of CIN diseases, according to age.*

Age	CIN I	CIN II	CIN III	Total
< 25	0	1	0	1
25-34	10	12	21	43
35-44	28	28	48	104
45-54	32	18	31	81
55-64	8	5	6	19
> 64	0	3	2	5
Subtotal	78	67	108	253

CIN I: cervical intraepithelial neoplasia I; CIN II:cervical intraepithelial neoplasia II; CIN III: cervical intraepithelial neoplasia III.

(3,897/14,991) and hr-HPV prevalence was 20.37% (3,054/14,991). The prevalence of HPV and hr-HPV according to age is shown Table 1 and in Figure 1. The prevalence of HPV and hr-HPV was highest in 55-64-year-old group and in the youngest group. There were significant difference in the age groups according to the prevalence of HPV ($\chi^2 = 35.793$, $p < 0.01$) and hr-HPV ($\chi^2 = 37.125$, $p < 0.01$).

The most prevalent genotype was HPV 52, which was detected in 683 (17.53%) out of the 3,897 HPV-positive samples. The second genotype was HPV 16, which was detected in 572 (14.68%). Genotype 58 was the third one, which was 13.06% (509/3,897), followed by unknown-risk HPV 81 and 53, with a prevalence of 12.21% (476/3,897) and 10.08% (393/3,897). HPV 68 was the sixth one, with a prevalence of 6.88% (268/3,897) and genotype 18 was the

Table 4. — *Detection of HPV and hr-HPV proportion of multiple-type infection according to cervical pathology status.*

Cervical Pathology Status	HPV positive		Single		Multiple infection (n,%)					
	Rate (n ,%)		Infection (n,%)		Co-infection		Tri-infection		Tetra- or more infection	
CIN I (78)	62	79.48	40	51.28	15	19.23	7	8.97	0	0
CIN II (67)	65	97.01	40	59.7	16	23.88	6	8.96	3	4.48
CIN III (108)	103	95.37	57	52.78	29	26.85	13	12.04	4	3.7
Total CIN (253)	230	90.9	137	54.15	60	23.71	23	10.28	7	2.77
χ^2	17.936		1.172		1.888					
					1.455		0.633		3.301	
p	< 0.001		0.557		0.389					
					0.483		0.729		0.192	
Cervical pathology status	Hr-HPV positive		Single		Multiple infection (n, %)					
	Rate (n, %)		Infection (n, %)		Co-infection		Tri-infection		Tetra- or more infection	
CIN I (78)	58	74.36	42	53.85	13	16.67	3	3.85	0	0
CIN II (67)	63	94.03	42	62.69	15	22.39	5	7.46	1	1.49
CIN III (108)	102	94.44	63	58.33	30	27.78	8	7.41	1	0.93
Total CIN (253)	223	88.14	147	58.1	58	22.92	16	6.32	2	0.79
χ^2	20.504		1.161		3.179		1.169		1.068	
p	< 0.01		0.56		0.204		0.557		0.586	

CIN I: cervical intraepithelial neoplasia I; CIN II: cervical intraepithelial neoplasia II; CIN III: cervical intraepithelial neoplasia III.

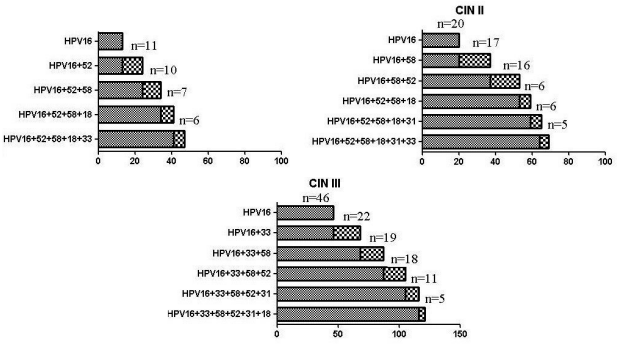

Figure 3. — Cumulative attribution rates of HPV types for cervical intraepithelial neoplasia (CIN).

seventh one at 6.54% (255/3,897) (Figure 2). In these hr-HPV genotypes, HPV 16 and 52 showed no difference between the status of different ages ($\chi^2 = 4.749$, $p = 0.447$; $\chi^2 = 5.536$, $p = 0.354$), and HPV 58, 18, and 68 was significant different in the age group, ($\chi^2 = 33.981$,$p < 0.01$; $\chi^2 = 15.569$, $p < 0.01$; $\chi^2 = 16.611$, $p < 0.01$), with HPV 58 highest in 55-64 group and > 64 group, HPV 18 highest in > 64 group, and HPV 68 highest in 55-64 group. Of these HPV-positive cases, 2,716 (18.12%) were single infection, and 1,181 (7.88%) were multiple infections. Among women with multiple infections, 803 women were infected with two HPV types, 257 women infected three HPV types, 73 cases with four HPV types, 32 cases with five HPV types, and 16 cases with more than five. The proportion of the multiple hr-HPV infections were higher in these women who were younger than 25 years and older than 55 years (Table 2); these differences were statistically significant ($\chi^2 = 43.668$, $p < 0.01$).

Of the 253 patients, the disease was found to be CIN I in 78 patients, CIN II in 67 patients, and CIN III in 108 patients (Table 3). The HPV positive rate was higher with CIN III and CIN II than CIN I (χ^2=17.936, $p < 0.001$) (Table 4), but no difference was seen between CIN II and CIN III ($\chi^2 = 0.291$, $p = 0.589$). The proportions of single- or multiple-type (co-, tri-, tetra-infection or more) infection are shown in Table 4. Infection with just one genotype or multiple-type infection seemed to have no relationship with the severity of pathology (χ^2=1.888, $p = 0.389$) (Table 4).

The four most prevalent types in CIN I with hr-HPV were HPV 16 (22.41%), HPV 52 (18.97%), HPV 58 (17.24%), and HPV 18 (12.07%); in CIN II they were HPV 16 (31.75%), HPV 58 (26.98%), HPV 52 (25.40%), and HPV18 (9.52%); in CIN III they were HPV 16 (45.10%), HPV 33 (21.57%), HPV 58(18.63%), and HPV 52 (17.65%). As shown in Figure 3, HPV16 plus 58 and 52 accounted for the majority of CIN with hr-HPV, with attribution rates of 54.84%, 84.61%, and 80.58 %, respectively. In CIN, other HPV subtypes in descending order were 81, 53, 68, 51, 56, 43, 35, 59, 42, 39, 11.83, 45, 73, and 82.

Of the samples from inpatients at the Department of Gynecology of Jiangsu University Hospital between July 2013 and July 2015, Stage IA1 CC in five patients, and Stage IA2 in two patients, Stage IIA in three patients, Stage IIIA in two patients, Stage IB1 in eight patients, Stage IIB in eight patients were histologically confirmed. However pre-operation HPV test confirmed only six patients with HPV 16 (four), HPV 52 (two), and HPV 18 (one).

Discussion

This is a study was performed in the North of Yangtze River Delta women for HPV subtype detection and distribution and its relationship with cervical precursors. The study of HPV prevalence and its subtype distribution will give guiding of public health policies and prevention measures. In different parts of world, there are many programs regarding HPV vaccinations for its prevention. Hence, it is important to characterize the types of HPV infections among specific populations regarding the need of vaccinations. The present data were much closer to not only the general population, but also to the carcinogenesis of HPV in the disease state.

In the present study, the population had overall 20.37% (3,054/14,991) prevalence of hr-HPV infections, and it was lower than the cities of Haikou, Chongqing, Jinan, Shenyang, Jilin, Tianjin, Shanghai, and Nanning. It was higher than Nanchang in the east of China, but similar to that described in Guiyang, Guangdong, Fuzhou, Chengdu, and Hangzhou [7], with no regional differences in China. In other areas of the world, the prevalence of hr-HPV infection was 27.4% from women seeking gynecological outpatient clinic in Greece [8] and 23% in the population of gynecological care in Turkey [9]. However, it was lower in Italy; a cross-sectional study carried out in 9,946 women showed an overall 14.8% of the hr-HPV infections [10], and in the UK and Ireland it was 13.2%) [11]. The different prevalences of the hr-HPV in the population of opportunistic hospital screening in various studies and the current study may be due to the differing populations, areas, and socio-economic, and cultural factors.

As in previous worldwide studies, it was found that the crude prevalence of hr-HPV cervical infection was highest in young women (< 35 years of age), decreasing in women of older age [12]. HPV incidence associated with younger age with a high peak of HPV infection suggests that younger, single women may have an increased possibility of encountering complicated sexual relationships [13]. However in the present study, the high prevalence was seen in the 55-64 age group, (24.86%) while the second peak was seen in the < 25 age group (24.01%). Multiple hr-HPV positivity was also more common in older women and younger women. It is may be that the younger women are less likely to have developed immunity to resist the HPV

infection. The women older than 50 years old are premenopausal or menopausal, who may be inclined to anxiety, sleeplessness, and so on, which may influence immunity.

In cervical diseases, multiple HPV infections occurred in 30.04% of women with CIN (20.51% for CIN I, 31.34% for CIN II, and 36.11% for CIN III). In the present study, infection with just one genotype or multiple-type infection seemed to show no relationship with the severity of pathology (χ^2=1.888, p = 0.389). To date, neither multiple-type infections, nor the type-specific contribution of HPV to CIN and CC progression is clear. Pista *et al.* found a significant association between multiple infections and disease severity [14]. Balbi *et al.* concluded that infection with simultaneous presence of multiple HPV types appears to be associated with a significantly increased risk of high grade lesions of CIN or invasive cancer, than with the presence of single viral infections [15]. The study from Trottier *et al.* showed that infections with multiple HPV types seem to act synergistically in cervical carcinogenesis[16]. However, Cuschieri *et al.* showed that women with multiple HPV infections at baseline were no more likely to develop cervical dyskaryosis than those with a single infection [17]. Hence, further efforts are needed to assure which conditions, with or without these HPV types, could induce CC.

In HPV subtypes, the present authors evaluated 17 hr subtypes, four lr subtypes, and two intermediate types 81 and HPV 53. The prevalence of HPV subtypes in descending order was 52, 16, 58, 81, 53, 68, 18, 51, 43, 56, 42, 35, 33, 6, 59, 31, 66, 11, 39, 45, 83, 73, and 82 (Figure 2). HPV 52 had the highest prevalence with 17.53% and HPV 16 had the second highest prevalence with 14.68% among HPV positive cases. Similar findings were seen in a study done in Zhejiang province of China where HPV 52 was the most prevalent subtype followed by HPV 16, then 58, 68, and 81 [18]. HPV16/52 showed no difference among the different age groups, but HPV58/18/68 was higher in the older group more than 55; this may provide some clues about the age of optimum inoculation, and vaccines protection period. In CIN I, II, and III lesions HPV 16 had the highest prevalence of 22.41%, 31.75%, and 45.10%, respectively. This distribution shows high carcinogenicity of HPV 16.

A survey of healthy women by the International Agency for Research on Cancer established that some genotypes were particularly prevalent in different continents; HPV 16 and HPV 18 were the two most common types in all regions (e.g., HPV 45 and 33 in Africa, HPV 33 and 31 in Europe, HPV 31, 33, and 45 in America, and HPV 58 and 52 in Asia [19]. There was no doubt that HPV 16 had overall importance in CIN. The relatively high contribution of HPV52 and HPV 58 to CC in East Asia was reported previously [18]. The current study further confirmed this fact and provided additional information. The present authors showed that HPV 58 and HPV 52 always ranked higher that CIN lesions. HPV 18 was not the most important, as it ranked seventh among HPV positive cases and ranked fourth in CIN I and CIN II, and sixth in CIN III. Of note, HPV 33 and31 ranked 13th and 16th among HPV positive population, but CIN III ranked second and fifth. Although HPV types 81 and 53 are classified as "possibly carcinogenic" because of limited evidence of their involvement in CC, in the present study they are also important in CIN.This suggested that these HPV types are vital to tumor progression in the study region. The present findings confirmed that the detection of lr-HPV types, mainly HPV 6 and 11 in CIN is a rare oncological event.

The development of a vaccine against HPV was a major breakthrough in science. HPV mainly infects the skin and mucosa, which is a small double-stranded DNA virus. The genome is divided into three regions: an early region (E1-7), late region (L1-2), and a long control region (LCR).The HPV vaccines are produced by recombinant DNA technology by incorporating L1or L2 capsid gene into a host cell (baculovirus/yeast) which replicates the proteins and then self-assemble into viral like particles (VLP). The VLP have similar size and shape with HPV virus which can stimulate CD4 + T cell mediated humoral immune response, produce neutralizing antibody of IgG, and protect vaccine recipients from the HPV subtype without infection and oncogenicity [20]. Two prophylactic HPV vaccines are commercially available to prevent infection with the HPV, the bivalent HPV vaccine (HPV 2 Cervarix) that protects against HPV genotypes 16 and 18 [21] and the quadrivalent vaccine (HPV 4, Gardasil) that protects against HPV genotypes 6, 11, 16 and 18 [22]. There is also a nine-valent vaccine which is in clinical trials, covering the quadrivalent vaccine, plus five additional types (31, 33, 45, 52, and 58) [23], which may be useful in the study regions.Although these vaccines can be very helpful in reducing CC mortality in the long term, their introduction in the present region in China is likely to meet several challenges. Studies indicated that the HPV genotypes: HPV 81, 53, and 68 were also found in a large proportion of women in the North of Yangtze River Delta women. Thus, a HPV vaccine covering HPV 52, 16, 58, or even better HPV 52, 16, 58, 81, 53, 68, 18, 33, and 31 should be designed or used in this region. Despite the marketing of HPV vaccines as the solution to CC, around the world there were some reports regarding discomfort after vaccination, therefore, the safety of the vaccines also need to be further researched. Which is the best way of immunity, the optimal dose, optimum inoculation, gender, age, and vaccines provide protection period, whether or not to perform cervical smear after inoculation, and how long it will take for a second class, are all problems that need to be solved. Other barriers for girls and women include high vaccine costs, inadequate delivery infrastructure, and lack of community engagement. Thus, HPV vaccines is also requires a public health solution.

The limitations of the present study include that it has a potential selection bias, as only one hospital and all the

cases were those who underwent a physical examination and visited the Cervical Disease Diagnosis and Treatment Center and Dermato-venereological Department of the Hospital. The selection was not random. In addition, the authors did not have sufficient data regarding HPV contribution in CIN and CC.

Conclusions

In conclusion, the present data showed except for HPV 16, low prevalence of HPV vaccine types of HPV 6 and 11, while a relatively high prevalence of HPV 58 and 52 in common women and those who were with precancerous lesions, which could serve as a guideline for future monitoring and preventing. Hence, a HPV vaccine covering HPV 52, 16, and 58, or even better HPV 52, 16, 58, 8, 53, 68, 18, 33, and 31 would be more useful in the forthcoming years in the North of Yangtze River Delta Women.Women with multiple HPV infections were no more likely to develop cervical precancers than those with a single infection.

Acknowledgement

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Corresponding Author:
YUMEI LI, M.D.
Dermatology and Venereology Department
Jiangsu University Hospital
Jie Fang Road 438
Zhenjiang City, Jiangsu Province (China)
e-mail: yumeili@ujs.edu.cn