Int J Clin Exp Pathol 2015;8(9):11901-11908
www.ijcep.com /ISSN:1936-2625/IJCEP0012479

Original Article

Distribution of HPV genotypes in Shanghai women

Suman Singh*, Qian Zhou*, Yunyun Yu, Xianghong Xu, Xiaojie Huang, Junwei Zhao, Lingfei Han, Kai Wang, Jing Sun, Fang Li

*Department of Gynecology, Shanghai First Maternity and Infant Hospital, Tongji University School of Medicine, Shanghai 200040, China. *Equal contributors.*

Received July 6, 2015; Accepted August 21, 2015; Epub September 1, 2015; Published September 15, 2015

Abstract: Objective: To study the Distribution of HPV genotypes in Shanghai women. Design: Cohort study. Setting: Shanghai First Maternity and Infant Hospital affiliated with Tongji University. Population: Patients those attended in the cervical disease diagnosis and treatment center of Shanghai First Maternity and Infant Hospital between January 2011 and December 2014. Methods: HPV GenoArray test kit (HybriBio Ltd) was used to perform HPV genotyping and was also used in DNA amplification and HybriBio's proprietary flow-through hybridization technique. Results: In this study, total patients analyzed were 4585. Among 4585 sample the HPV positive patients were 1460 i.e. 31.84% in total. On the basis of pathological report normal were 1358, with inflammation 2441, with low grade lesion were 399, high grade lesion were 353, CIN were 19 and cervical carcinoma were 15. Among normal HPV positive were 215 (15.8%), among inflammation HPV positive were 735 (30.11%). HPV positive in low grade lesion were 353 i.e. 59.77%. In high grade lesion 211 were HPV positive among 272 (68.17%). The percentage of HPV positive was 73.68% i.e. 14 out of 19 patient in cervical carcinoma in situ. 13 patient out of 15 i.e. 86.67% of Cervical carcinoma were HPV positive. Among all percentage of HPV positive was high among cervical carcinoma then cervical carcinoma in situ then high grade lesion in decreasing fashion to low grade lesion and in normal. Highest prevalence i.e. 22.67% is of HPV 52 subtype and HPV 16 has second highest prevalence with 17.67% among HPV positive cases. Sensitivity of TCT detection is 71.6%. Specificity of TCT detection is 79.6%. Sensitivity of HPV-DNA detection is 65.2%. Specificity of HPV-DNA detection is 78.2%. Conclusion: HPV is one of major health concern in shanghai having high prevalence rate in comparison to other part of china and other part of world. This has implications for the future cervical cancer burden and the priority to be given to prevent cervical cancer in Shanghai, especially, given the promising efficacy of prophylactic vaccines against HPV52, 16 and 58. This study also shows high sensitivity and specificity of TCT and HPV-DNA detection.

Keywords: Cervical intraepithelial neoplasia, HPV infection, HPV distribution in shanghai, HPV genotype, HPV subtypes, TCT sensitivity, TCT specificity

Introduction

Human papillomavirus (HPV) is the most common sexually transmitted infections among women worldwide. Cervical pre-malignant lesions and cervical carcinoma which is one of the most common cancers among women worldwide is associated with Persistent infection caused by high-risk HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68). During the sexual life most of the women are infected with one or more (mixed) HPV genotypes [1]. The HPV infection and cervical cancer association is documented widely. The majority of cervical cancers are the consequence of persistent infection by high risk HPV genotypes [2]. The association of HPV infection with cervical cancer and its precursor lesions has been well established [3]. Recent studies have shown that HPV DNA can be found in 99.7% of all cervical cancers while most common subtypes of HPV types are 16, 18, 45, and 31. According to the etiologic role in progression to cervical cancer, HPV subtypes are classified as high risk includes 15 HPV genotypes, probable high risk contains three genotypes (26, 53, and 66), low risk contains 12 genotypes (such as 6, 11 and others frequently detected in benign lesions such as condyloma accuminata) and undetermined risk contains three genotypes (34, 5, and 83) [4]. The high risk types HPV-16 and HPV-18 are responsible for approximately 70% of cervical cancers [5] worldwide, HPV-16 is present in 45% and HPV-18 in 16% of cervical lesions. Genotypes 31 and 45 represent for another 10% [6]. Although

Table 1. HPV Prevalence among Women participating in population-based Cervical screening studies

Study year	Location	Number (N)	Age	Target HPV types	Overall Prevalence
1999	Xiangyuan	1997	35-45	13HR-HPV	18.2
2001-2002	Yangcheng and xiangyuan	8798	35-50	13HR-HPV	23.6
2001-2002	Xiangyuan and yangcheng Shanxi	9683	30-50	13HR-HPV	27.5
2004	Yangchen	745	15-59	13HR-HPV	16
2004	Xiushui Jiangxi	2432	30-49	13HR-HPV	18.5
2004-2005	Shenyang	685	15-59	HR-HPV&30 LR-HPV	16.8
2004-2005	Yangcheng	662	15-59	HR-HPV&30 LR-HPV	14.8
2004-2005	Shenzhen	1027	15-59	HR-HPV&30 LR-HPV	16.6
1999-2008	Rural Areas And urban areas	29579	15-59	13 HR-HPV	18
2006-2009	Beijing	6185	25-54	13 HR-HPV	9.9
2009	Jiangsu	316	18-25	13 HR-HPV	17.1

HR-HPV genotypes 16 and 18 causes 70% of ICC cases and 50% of CIN2-3 according to the general consensus, HPV-genotype distribution of high-risk HPV types may vary by age [7] race/ethnicity [8], which is also linked to socioeconomic status [9] and geographic location [10, 11].

Cancer of the cervix is the third most common cancer in women worldwide, with an estimated incidence of 529,000 cases and 274,000 deaths occurring in 2008 [12]. More than 85% of the cases occur in developing countries such as China. The "two peak" pattern pertained to both rural and urban women. The crude HR-HPV prevalence was seen to peak among urban women aged 15-24 years (18.7%) and among women older than 40 years of age (16.0%). Among rural women, it peaked at age 15-24 years (16.2%) and 35-39 years (18.6%) [13]. This pattern differs from that observed in women from Western countries, among whom, HPV prevalence peaked only at their mid-twenties, then steadily decline as age increases [14]. The first peak in China may be due to the fact that younger women are more sexually active and more likely to have multiple partners, especially in urban areas [15]. These findings suggest that HPV vaccination of women before the completion of national compulsory education between the ages of 13 and 15 years is likely to contribute to the prevention of HPV infection and cervical cancer in China. Reasons for the second peak in Chinese women are not well understood; viral persistent infection may be one of the explanations [16] or reactivation of a latent HPV infection.

Several multi-centre, hospital-/population-based studies published over a 12-year period (2001-2012) including the first population-based HPV study published in China is shown in **Table 1** [17].

Cervical intraepithelial neoplasia (CIN) is a group of precancerous lesions, which is closely related to cervical cancer [18]. If women with high-grade CIN are not treated, they are at very high risk of acquiring cervical cancer [19].

Material and methods

Ethics statement

This project has been approved by the Scientific and Ethical Committee of the Shanghai First Maternity and Infant Hospital affiliated with Tongji University (permit number: K08-018). All of the samples and data were collected with a written informed consent provided by the participants.

Study population

Patients those attended in the cervical disease diagnosis and treatment center of Shanghai First Maternity and Infant Hospital between January 2011 and December 2014 were recruited. Patients with pregnancy, known malignancy or immunosuppressive diseases or receiving immunosuppressive therapy were excluded from the study. Patients underwent cervical examination, pelvic examination, and cytological testing with Thin prep cytologic test (TCT). Suspected case went for colposcopic examination and Leep biopsy.

Cervical cells were collected with a cytobrush from ectocervix and endocervix of the uterus of every woman by cervical scrapings. Smeared cell slides were prepared from scraping for thinprep liquid-based cytology test and the remaining samples were suspended in PBS and stored

Table 2. Prevalence of HPV

HPV types	Normal	Inflammation	Low grade lesion	High grade lesion	Cervical carcinoma in situ	Cervical carcinoma	subtotal	percentage
Subtotal	1358	2441	353	399	19	15	4585	
HPV+ (%)	215 (15.83)	735 (30.11)	211 (59.77)	272 (68.17)	14 (73.68)	13 (86.67)	1460	31.84%
HPV- (%)	1143 (84.17)	1706 (69.89)	142 (40.23)	127 (31.83)	5 (26.32)	2 (13.33)	3125	68.15%
P value		9.0808E-22	1.28863E-55	2.48763E-77	2.87833E-07	3.4532E-06		

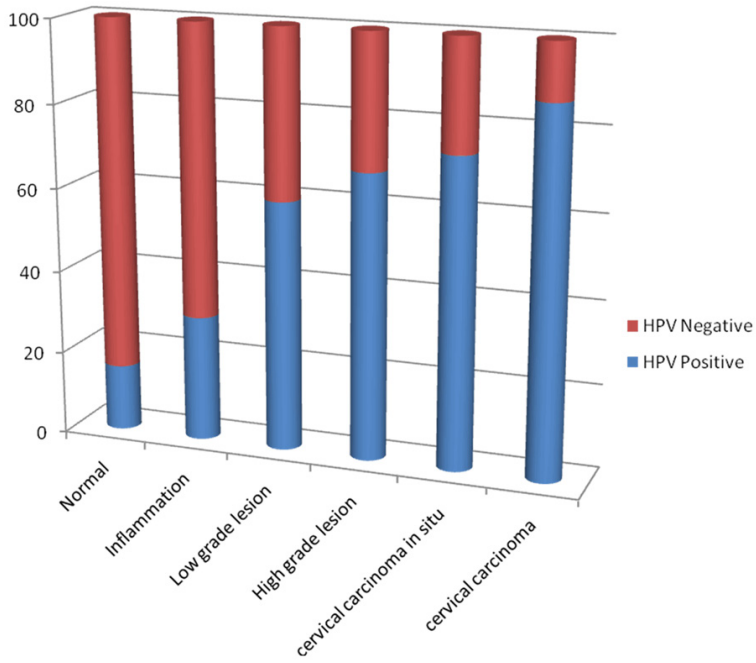

Figure 1. HPV prevalence in different cervical pathology.

at -70°C for DNA extraction. The HPV detection and pathological diagnosis were done separately.

HPV genotyping

The HPV GenoArray test kit (HybriBio Ltd) was used to perform Human papillomavirus genotyping. It was used in both DNA amplification and HybriBio's proprietary flow-through hybridization technique. HPV Blot contains 21 types of genotypes, including 5 low-risk types (6, 11, 42, 43, and 44), 14 high-risk types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68), and 2 intermediate-risk types (CP8304 and 53) common in china people. Absence of HPV DNA contamination was confirmed by HPV L1 and an internal control of the human A globin in each reaction.

Cytology

Thinprep cytologic test (TCT) was performed with a special cytobrush to collect cervical exfoliated cells. These cells were stored in the PreservCyt. All specimens were divided into negative for intraepithelial lesion or malignancy (NILM), atypical squamous cells of undetermined significance (ASCUS), atypical squamous cells-high grade (ASC-H), low-grade squamous intraepithelial lesion (LSIL) and high-grade group consisted of high-grade squamous intraepithelial lesion (HSIL) according to the Bethesda system.

Reverse transcription-polymerase chain reaction

Cervical exfoliated cells were collected with an endocervical cytobrush and stored in normal saline. Trizol reagent was used for RNA extraction according to the protocol of manufacturer protocol. The concentration of RNA was detected by SmartSpec plus. RNA was reverse transcription into cDNA according to 500 ng/μl RNA using PrimeScriptTMRT reagent Kit (code: DRR037 s, 100 reactions, TakaRa) following the manufacturer's protocol. The PCR was performed in a 25 μl reaction volume including 1 μl cDNA, 12.5 μl premix Taq (code: D331, 500 μl, TaKaRa), 0.5 μl primer forward, 0.5 μl primer right and 10.5 μl dH_2O. Primer sequences were as follow: IFN-γ: Forward 5'-ATGAAATATACAAGTTATATCTTGGCT-3' Reverse 5'-GCGACAGTTCAGCCATCACTTG-3' (420 bp) β-action: Forward 5'-ATGGGTCAGAAGGATTCCTATGTG-3' Reverse 5'-CTTCATGAGGTGTCAGTCAGGTC-3' (434 bp). The amplification conditions: after preheating at 95°C for 2 min, 35 cycles of denaturation at 95°C for 30 sec, annealing at 55°C for 1 min, extension at 72°C for 1 min. The PCR products were electrophoresed on 2% agarose gel and visualized by an Eagle Eye analyser [20].

Table 3. Prevalence of HPV subtypes

HPV types	Normal	Inflammation	Low grade lesion	High grade lesion	Cervical carcinoma in situ	Cervical carcinoma	Total	Percentage
Subtotal	1358	2441	353	399	19	15		
HPV+HR								
16	23 (10.70)	102 (13.88)	72 (34.12)	42 (15.44)	9 (64.29)	10 (76.92)	258	17.67%
18	15 (6.98)	46 (6.26)	13 (6.16)	20 (7.35)	0 (0)	0 (0)	94	6.43%
31	14 (6.51)	56 (7.62)	20 (9.48)	16 (5.88)	1 (7.14)	0 (0)	107	7.32%
33	7 (3.26)	42 (5.71)	19 (9)	19 (6.99)	1 (7.14)	1 (7.69)	89	6.09
35	4 (1.86)	7 (0.95)	3 (1.42)	4 (1.47)	0 (0)	0 (0)	18	1.23%
39	17 (7.91)	46 (6.26)	7 (3.32)	21 (7.72)	1 (7.14)	1 (7.69)	93	6.36%
45	2 (0.93)	13 (1.77)	2 (0.95)	2 (0.74)	0 (0)	1 (7.69)	20	1.36%
51	8 (3.72)	26 (3.54)	9 (4.27)	14 (5.15)	1 (7.14)	1 (7.69)	59	4.04%
52	47 (21.86)	157 (21.36)	53 (25.12)	74 (27.21)	0 (0)	0 (0)	331	22.67%
56	8 (3.72)	36 (4.9)	3 (1.42)	14 (5.15)	1 (7.14)	0 (0)	62	4.24%
58	32 (14.88)	114 (15.51)	50 (23.7)	55 (20.22)	2 (14.29)	1 (7.69)	254	17.39%
59	7 (3.26)	21 (2.86)	7 (3.32)	8 (2.94)	0 (0)	1 (7.69)	44	3.01%
66	8 (3.72)	39 (5.31)	7 (3.32)	15 (5.51)	0 (0)	2 (15.38)	71	4.86%
68	20 (9.30)	43 (5.85)	8 (3.79)	18 (6.62)	1 (7.14)	0 (0)	90	6.16%
HPV+LR								
6	12 (5.58)	28 (3.81)	5 (2.37)	7 (2.57)	0 (0)	0 (0)	52	3.56%
11	7 (3.26)	16 (2.18)	4 (1.9)	11 (4.04)	0 (0)	0 (0)	38	2.60%
42	1 (0.47)	11 (1.5)	1 (0.47)	2 (0.74)	0 (0)	0 (0)	15	1.02%
43	0 (0.00)	1 (0.14)	0 (0)	0 (0)	0 (0)	0 (0)	1	0.06%
44	7(3.26)	8 (1.09)	1 (0.47)	3 (1.1)	0 (0)	0 (0)	19	1.30%
HPV+IR								
CP8304	31 (14.42)	105 (14.29)	14 (6.64)	32 (11.76)	0 (0)	1 (7.69)	183	12.53%
53	30 (13.95)	87 (11.84)	11 (5.21)	34 (12.5)	0 (0)	0 (0)	162	11.09%

HR: high risk, LR: low risk, IR: Intermediate.

Statistical analysis

The data were processed and analyzed with the SPSS (version 13.0; Tongji University, China). Statistical analyses were done with binary and multinomial logistic regression.

Results

Total patient analyzed in this study was 4585. Among all according to pathological report normal were 1358, with inflammation 2441, with low grade lesion were 353, high grade lesion were 399, CIN were 19 and cervical carcinoma were 15 shown in **Table 2**. Among normal HPV positive were 215 (15.8%), among inflammation HPV positive were 735 (30.11%). HPV positive in low grade lesion were 211 (59.77%). In high grade lesion 272 were HPV positive among 399 i.e. 68.17%. In cervical carcinoma in situ 73.68% patients were HPV positive i.e. 14 out of 19. 13 patient out of 15 i.e. 86.67% of Cervical carcinoma were HPV positive. HPV prevalence is highest in cervical carcinoma (**Figure 1**). 1460 patients were HPV positive among 4585 sample i.e. 31.84% cases were HPV positive in total. Among all percentage of HPV positive was high among cervical carcinoma then cervical carcinoma in situ then high grade lesion in decreasing fashion to low grade lesion and in normal. There was no significant difference in between HPV positive and negative cases in any subtypes (P value > 0.001 in all subtypes) so it can't be stated that any of the lesion described was only due to HPV but it is one of the main factor associated with cervical lesion.

We saw HPV subtype positivity in different cases. It was found that HPV 52 had higher prevalence than other among normal, inflammation and high grade clinical subtypes, while HPV 16 had higher prevalence in low grade lesion (34.12%), CIN (64.29%) and cervical carcinoma (76.92%) shown in **Table 3**. Among low

Table 4. Age wise distribution of cervical pathology

Age (years)	Normal	Inflammation	Low grade lesion	High grade lesion	Cervical carcinoma in situ	Cervical carcinoma
< 25	60	74	5	15	0	0
25-34	546	914	104	168	4	1
35-44	362	734	121	100	8	4
45-54	239	429	86	63	5	7
55-64	116	230	31	45	1	3
≥ 65	35	60	6	8	1	0
subtotal	1358	2441	353	399	19	15

Table 5. Age wise distribution of HPV positive

Age (years)	Subtotal	HPV-	HPV+	HPV+%	*P* value
< 25	165	103	62	37.6%	
25-34	1844	1264	580	31.5%	0.303
35-44	1396	982	414	29.7%	0.986
45-54	868	605	263	30.3%	0.678
55-64	446	277	169	37.9%	0.791
≥ 65	111	76	35	31.5%	0.214
subtotal		3307	1523		
total	4830				

risk HPV, HPV 6 and 11 have high prevalence. While only high risk HPV were present in CIN and cervical carcinoma which shows higher chance of association between High risk HPV and CIN & cervical carcinoma.

If we see the pathological findings (shown in **Table 4**) cervical carcinoma is maximum in 45-54 years while cervical carcinoma in situ is having maximum prevalence in 35-44 years. High grade lesion is maximum in 25-34 while low grade lesion is maximum at 35-44 years and inflammation is maximum at 25-34 years. Normal population HPV prevalence is maximum at 25-34 years.

In age wise distribution, it is seen that higher prevalence among < 25 and 55-64 age group. This explains the nature of HPV infection which is common in sexually active women in reproductive age group shown in **Table 5**.

On comparison of TCT and biopsy results, the sensitivity of TCT was 76.1% and the specificity was 79.6% (**Table 6**); False negative rate of TCT detection = (58+90+1+1+0+0)/(309+200+19) = 0.284 i.e.28.4%; Sensitivity of TCT detection = 1-0.284 = 0.716 i.e.71.6%; False positive rate of TCT detection = (2+8+116+341+9+61+2+6+0+9)/(1052+1669) = 0.204 i.e. 20.4%; Specificity of TCT detection = 1-0.204 = 0.796 i.e. 79.6%; According to **Table 7**. false negative rate of HPV-DNA detection = (75+106+3)/(309+200+19) = 0.348 i.e. 34.8%; Sensitivity of HPV-DNA detection = 1-0.348 = 0.652 i.e. 65.2%; False positive rate of HPV-DNA detection = (134+459)/(1052+1669) = 0.218 i.e. 21.8%; Specificity of HPV-DNA detection = 1-0.218 = 0.782 i.e.78.2%.

Discussion

This is one of the largest study done in shanghai women for HPV detection and its subtype distribution. As HPV is the most common infection transmitted sexually and due to its high prevalence, its matter of concern for health. There is lots of program regarding HPV vaccination for HPV prophylaxis in different part of world with different types of strain. The study of HPV prevalence and its subtype distribution will give clue regarding need of routine vaccination and the types of the HPV strain to be used in vaccination.

The estimated crude and adjusted HPV prevalence among women with normal cytological findings worldwide were 7.2% and 11.7%, respectively. Sub-Saharan African regions (24.0%), Latin America and the Caribbean (16.1%), Eastern Europe (14.2%), and Southeastern Asia (14.0%) had the highest prevalence [21]. While in this study the HPV prevalence with normal cytological findings is 15.83% which is slightly higher than the world's total prevalence but similar to Latin America, eastern Europe and southern Asia but slightly higher than the other study in china done in Shenyang city where it was 13.6% while the overall HPV prevalence is quite high 31.84% as compared to 16.8% in Li et al [22] and 22.5% in Wu D et al [23]. But the total prevalence is lower than the study done in Beijing which has 53.7% overall prevalence in Ding et al [24]. Overall HPV infection prevalence was 29.1% in a study done in the shanghai in Zhang et al [25] which is similar to our study which shows accuracy of our study.

Table 6. Comparison between TCT and biopsy report for pathological findings

TCT results	Biopsy results				
	Normal	Inflammation	Low grade lesion	High grade lesion	Cervical carcinoma
NILM	923	1219	58	90	1
Inflammation	0	25	0	1	0
AGC	2	8	1	0	0
ASC-US	116	341	63	119	9
LSIL	9	61	34	97	1
ASC-H	2	6	6	1	1
HSIL	0	9	38	1	7
subtotal	1052	1669	200	309	19

Table 7. HPV positivity among biopsy cases

HPV results	Biopsy results				
	Normal	Inflammation	Low grade lesion	High grade lesion	Cervical carcinoma
HPV high risk-	918	1210	75	106	3
HPV high risk+	134	459	125	203	16
subtotal	1052	1669	200	309	19

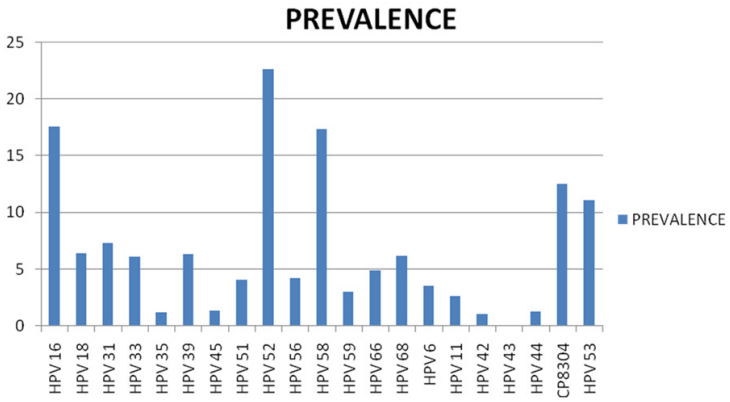

Figure 2. Prevalence of HPV subtypes.

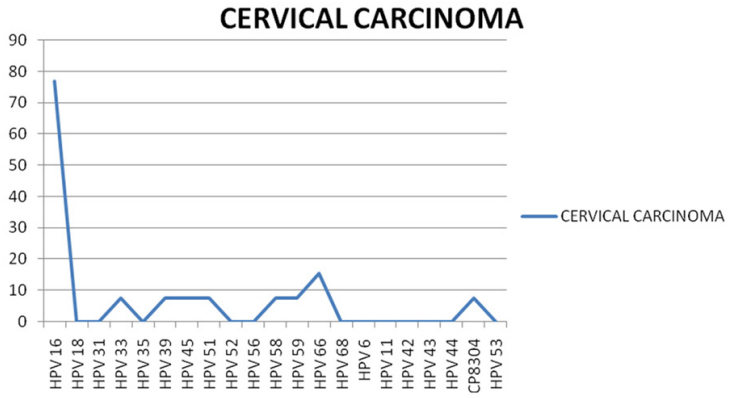

Figure 3. Distribution of HPV subtypes in cervical carcinoma.

In all world regions, HPV prevalence was highest in women younger than 35 years of age, decreasing in women of older age. In Africa, the Americas, and Europe, a clear second peak of HPV prevalence was observed in women aged 45 years or older [26]. The HPV prevalence in women 18-20 years old it was 54.4% (31/57), the highest among all age groups. After declining rapidly, HPV prevalence stabilized at about 30.0% in women aged 30 and older in Zhang et al [25]. But in this study if we see the age wise distribution, the high prevalence is seen in < 25 years i.e. 37.6% while the second peak is observed in age 55-64 i.e. 37.9%. 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 [27].

In HPV subtypes, we evaluated 14 HR (high-risk) subtypes, 5 LR (low risk subtypes) and 2 intermediate types CP8304 & HPV 53. HPV 52 is having highest prevalence with 22.67% and HPV 16 has second highest prevalence with 17.67% among HPV positive cases. But in cervical carcinoma and carcinoma in situ and low grade lesion HPV 16 has highest prevalence of 76.92%, 64.29% and 34.12% respectively. While HPV 52 has high prevalence in normal, inflammation and high grade lesion with 21%, 21% and 27% respectively. This distribution shows high carcinogenicity of HPV 16 and low carcinogenicity of HPV 52. In high risk type the prevalence of HPV subtypes in descending order is 52, 16, 58, 53, 31, 18, 39, 68, 33, 66, 56, 51, 59, 45 and 35 (**Figure 2**). While in low risk HPV subtypes in descending order of prevalence is CP8304, 6, 11, 44, 42 and 43.

Similar findings were seen in a study done in Fujian province china where HPV 52 was the most prevalent (23.1%) subtypes followed by HPV 16, 18 and 33 [23]. The HPV subtypes associated with cervical carcinoma are 16, 33, 39, 45, 51, 59, 66 and CP8304 (**Figure 3**). Those associated with CIN are 16, 58, 31, 33, 39, 51, 56 and 68.

We have also analyzed the high sensitivity, specificity, positive predictive value and negative predictive value of TCT test but is less if compared to other study which shows higher detection rate with value 80.0%, 63.2%, 16.0%, 97.3% respectively [28].

The limitation of our study is this includes only hospital based survey as we have taken all the cases visited to the cervical disease diagnosis and treatment center of Shanghai First Maternity and Infant Hospital. The selection was not random and was not specifically categorized.

Conclusion

HPV is one of major health concern in shanghai having high prevalence rate in comparison to other part of world and other part of China. Rapid industrialization, urbanization and rapid changes in sexual behavior may be the contribution factor for high HPV prevalence. This has implications for the future cervical cancer burden and the priority to be given to preventing cervical cancer in Shanghai, especially, given the promising efficacy of prophylactic vaccines against HPV 52, 16 and 58. This study also analyzed high sensitivity, specificity, positive predictive value and negative predictive value of TCT and HPV-DNA detection.

Acknowledgements

This work was supported by grants from the Science and Technology Committee of Shanghai (NO.15411967700, F.L.), Shanghai Hospital Development Centre (SHDC-12012315), Shanghai Natural Science Foundation (NO.15ZR1433300) and National Natural Science Foundation of China (NO.81572546).

Disclosure of conflict of interest

None.

Address correspondence to: Drs. Fang Li and Jing Sun, Department of Gynecology, Shanghai First Maternity and Infant Hospital, Tongji University School of Medicine, Shanghai 200040, China. Tel: +86-21-20261136; Fax: +86-20-50730190; E-mail: fang_li@tongji.edu.cn

References

[1] Schiffman M, Castle PE, Jeronimo J, Rodriguez AC, Wacholder S. Human papillomavirus and cervical cancer. Lancet 2007; 370: 890-907.

[2] Ho GY, Burk RD, Klein S, Kadish AS, Chang CJ, Palan P, Basu J, Tachezy R, Lewis R, Romney S. Persistent genital human papillomavirus infection as a risk factor for persistent cervical dysplasia. J Natl Cancer Inst 1995; 87: 1365-71.

[3] Walboomers JM, Jacobs MV, Manos MM, Bosch FX, Kummer JA, Shah KV, Snijders PJ, Peto J, Meijer CJ, Muñoz N. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol 1999; 189: 12-9.

[4] Munoz N, Bosch FX, de Sanjose S, Herrero R, Castellsague X, Shah KV, Snijders PJ, Meijer CJ; International Agency for Research on Cancer Multicenter Cervical Cancer Study Group. Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Engl J Med 2003; 348: 518-27.

[5] Lacey CJ, Lowndes CM, Shah KV. Chapter 4: Burden and management of non-cancerous HPV-related conditions: HPV-6/11 disease. Vaccine 2006; 31: 35-41.

[6] de Sanjose S, Quint WG, Alemany L, Geraets DT, Klaustermeier JE, Lloveras B, Tous S, Felix A, Bravo LE, Shin HR, Vallejos CS, de Ruiz PA, Lima MA, Guimera N, Clavero O, Alejo M, Llombart-Bosch A, Cheng-Yang C, Tatti SA, Kasamatsu E, Iljazovic E, Odida M, Prado R, Seoud M, Grce M, Usubutun A, Jain A, Suarez GA, Lombardi LE, Banjo A, Menéndez C, Domingo EJ, Velasco J, Nessa A, Chichareon SC, Qiao YL, Lerma E, Garland SM, Sasagawa T, Ferrera A, Hammouda D, Mariani L, Pelayo A, Steiner I, Oliva E, Meijer CJ, Al-Jassar WF, Cruz E, Wright TC, Puras A, Llave CL, Tzardi M, Agorastos T, Garcia-Barriola V, Clavel C, Ordi J, Andújar M, Castellsagué X, Sánchez GI, Nowakowski AM, Bornstein J, Muñoz N, Bosch FX; Retrospective International Survey and HPV Time Trends Study Group. Human papillomavirus genotype attribution in invasive cervical cancer: a retrospective cross-sectional worldwide study. Lancet Oncol 2010; 11: 1048-56.

[7] Barnholtz-Sloan J, Patel N, Rollison D, Kortepeter K, MacKinnon J, Giuliano A. Incidence trends of invasive cervical cancer in the United States by combined race and ethnicity. Cancer Causes Control 2009; 20: 1129-38.

[8] Hariri S, Unger ER, Powell SE, Bauer HM, Bennett NM, Bloch KC, Niccolai LM, Schafer S, Steinau M, Markowitz LE; HPV-IMPACT Working

Group. Human papillomavirus genotypes in high-grade cervical lesions in the United States. J Infect Dis 2012; 206: 1878-86.
[9] Niccolai LM, Russ C, Julian PJ, Hariri S, Sinard J, Meek JI, McBride V, Markowitz LE, Unger ER, Hadler JL, Sosa LE. Individual and geographic disparities in human papillomavirus types 16/18 in high-grade cervical lesions: Associations with race, ethnicity, and poverty. Cancer 2013; 119: 3052-8.
[10] Smith JS, Lindsay L, Hoots B, Keys J, Franceschi S, Winer R, Clifford GM. Human papillomavirus type distribution in invasive cervical cancer and high-grade cervical lesions: a meta-analysis update. Int J Cancer 2007; 121: 621-32.
[11] Vidal AC, Murphy SK, Hernandez BY, Vasquez B, Bartlett JA, Oneko O, Mlay P, Obure J, Overcash F, Smith JS, van der Kolk M, Hoyo C. Distribution of HPV genotypes in cervical intraepithelial lesions and cervical cancer in Tanzanian women. Infect Agent Cancer 2011; 6: 1750-9378.
[12] Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer 2010; 127: 2893-917.
[13] Zhao FH, Lewkowitz AK, Hu SY, Chen F, Li LY, Zhang QM, Wu RF, Li CQ, Wei LH, Xu AD, Zhang WH, Pan QJ, Zhang X, Belinson JL, Sellors JW, Smith JS, Qiao YL, Franceschi S. Prevalence of human papillomavirus and cervical intraepithelial neoplasia in China: a pooled analysis of 17 population-based studies. Int J Cancer 2012; 131: 2929-38.
[14] Moscicki AB, Schiffman M, Kjaer S, Villa LL. Chapter 5: Updating the natural history of HPV and anogenital cancer. Vaccine 2006; 31: 42-51.
[15] Zhao FH, Tiggelaar SM, Hu SY, Xu LN, Hong Y, Niyazi M, Gao XH, Ju LR, Zhang LQ, Feng XX, Duan XZ, Song XL, Wang J, Yang Y, Li CQ, Liu JH, Liu JH, Lu YB, Li L, Zhou Q, Liu JF, Zhao N, Schmidt JE, Qiao YL. A multi-center survey of age of sexual debut and sexual behavior in Chinese women: suggestions for optimal age of human papillomavirus vaccination in China. Cancer Epidemiol 2012; 36: 384-90.
[16] Castle PE, Schiffman M, Herrero R, Hildesheim A, Rodriguez AC, Bratti MC, Sherman ME, Wacholder S, Tarone R, Burk RD. A prospective study of age trends in cervical human papillomavirus acquisition and persistence in Guanacaste, Costa Rica. J Infect Dis 2005; 191: 1808-16.
[17] Li J, Huang R, Schmidt JE, Qiao YL. Epidemiological features of Human Papillomavirus (HPV) infection among women living in Mainland China. Asian Pac J Cancer Prev 2013; 14: 4015-23.
[18] Giorgi-Rossi P, Franceschi S, Ronco G. HPV prevalence and accuracy of HPV testing to detect high-grade cervical intraepithelial neoplasia. Int J Cancer 2012; 130: 1387-94.
[19] Nam K, Chung S, Kim J, Jeon S, Bae D. Factors associated with HPV persistence after conization in patients with negative margins. J Gynecol Oncol 2009; 20: 91-5.
[20] Khatissian E, Monceaux V, Cumont MC, Montagnier L, Hurtrel B, Chakrabarti L. Interferon-gamma expression in macaque lymph nodes during primary infection with simian immunodeficiency virus. Cytokine 1996; 8: 844-52.
[21] Bruni L, Diaz M, Castellsague X, Ferrer E, Bosch FX, de Sanjose S. Cervical human papillomavirus prevalence in 5 continents: meta-analysis of 1 million women with normal cytological findings. J Infect Dis 2010; 202: 1789-99.
[22] Li LK, Dai M, Clifford GM, Yao WQ, Arslan A, Li N, Shi JF, Snijders PJ, Meijer CJ, Qiao YL, Franceschi S. Human papillomavirus infection in Shenyang City, People's Republic of China: A population-based study. Br J Cancer 2006; 95: 1593-7.
[23] Wu D, Cai L, Huang M, Zheng Y, Yu J. Prevalence of genital human papillomavirus infection and genotypes among women from Fujian province, PR China. Eur J Obstet Gynecol Reprod Biol 2010; 151: 86-90.
[24] Ding X, Liu Z, Su J, Yan D, Sun W, Zeng Z. Human papillomavirus type-specific prevalence in women referred for colposcopic examination in Beijing. J Med Virol 2014; 86: 1937-43.
[25] Zhang WY, Xue YZ, Chen M, Han L, Luo M. Prevalence of high-risk human papillomavirus infection in different cervical lesion among organized health-examination women in Shanghai, China. Chin Med J 2008; 121: 1578-82.
[26] de Sanjose S, Diaz M, Castellsague X, Clifford G, Bruni L, Munoz N, Bosch FX. Worldwide prevalence and genotype distribution of cervical human papillomavirus DNA in women with normal cytology: a meta-analysis. Lancet Infect Dis 2007; 7: 453-9.
[27] Vaccarella S, Franceschi S, Herrero R, Muñoz N, Snijders PJ, Clifford GM, Smith JS, Lazcano-Ponce E, Sukvirach S, Shin HR, de Sanjosé S, Molano M, Matos E, Ferreccio C, Anh PT, Thomas JO, Meijer CJ; IARC HPV Prevalence Surveys Study Group. Sexual behavior, condom use, and human papillomavirus: pooled analysis of the IARC human papillomavirus prevalence surveys. Cancer Epidemiol Biomarkers Prev 2006; 15: 326-33.
[28] Chen H, Shu HM, Chang ZL, Wang ZF, Yao HH, Zhu HM, Lu TM, Ma QY, Yang BL. Efficacy of Pap test in combination with ThinPrep cytological test in screening for cervical cancer. Asian Pac J Cancer Prev 2012; 13: 1651-5.