Distribution of human papillomavirus genotypes (2014–2016) in women with genital warts at a sexually transmitted disease clinic in Beijing, China

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Aim: The aim of this study was to analyze the distribution of human papillomavirus (HPV) genotypes among women with genital warts in the Beijing region of China. **Methods:** A total of 350 women diagnosed with genital warts between 2014 and 2016 were analyzed by real-time polymerase chain reaction (PCR) to test for high-risk and low-risk HPV subtypes. **Results:** The results show that 52.6% (184/350) of cases were positive for HPV6+11. The three most common high-risk HPVs detected were HPV52, HPV16 and HPV58, detected in 12.0% (42/350), 10.6% (37/350) and 10.0% (35/350) of all cases, respectively. **Conclusion:** These results indicate that vaccines targeting HPV subtypes 6, 11, 16, 52 and 58 would have the greatest impact among sexually active women living in Beijing.

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Human papillomaviruses (HPVs) are small double-stranded DNA viruses that belong to the family of papillomavirus [1]. There are as many as 170 HPV genotypes have been identified [2], and approximately 40 kinds of these types specially infect the anogenital tract [3]. Genital warts are evident skin or mucosal growths in the anogenital area display as flat, papular or pedunculated growths on the genital mucosa [4]. Genital warts are one of the most common sexually transmitted diseases with a high prevalence (0.5%) in Asia [5,6]. The disease results from infection with ‘low-risk’ HPV subtypes [7]. Infection with ‘high-risk’ HPV is associated with the development of precancerous lesions [8]. Treatments on genital warts are mainly divided into medications and surgery, medications include imiquimod, podophyllin, podofilox and trichloroacetic acid, surgery include freezing with liquid nitrogen, electrocautery, surgical excision and laser treatments [9,10]. Although the short-time effect of above treatment is good, the transmission rate of the related HPV infection is as high as 65%, with a high risk of recurrence [7]. Thus, effective prevention against HPV transmission and infection is essential. Accordingly, many countries have implemented HPV vaccination programs.

A recent study by Chang *et al.* [11] indicates that the distribution of HPV subtypes differs among various regions throughout China. Comparison of other studies reporting on HPV prevalence in China indicates wide geographic variability in subtype distribution [12], thus undermining the selection of the most effective vaccine. Up to date, several vaccines have been developed to prevent HPV infection. Gardasil is a quadrivalent vaccine containing four genotypes, namely 16, 18, 6 and 11; and Cervarix (GSK) is a bivalent vaccine containing 16 and 18. In addition to providing excellent protection against the HPV types contained in the vaccine, Gardasil has also demonstrated significant cross-protection to other oncogenic types in both naive and previously infected women [13].

In order to identify the distribution of HPV subtypes in the Beijing area, samples collected from women with genital warts treated at a sexually transmitted disease clinic were examined. The results will contribute to the data

Table 1. Human papillomavirus genotypes frequencies in genital warts.

Genotype	Single infection	Multiple infection					Total	% (95% CI)
		2	3	4	5	6		
No.	127	63	32	16	9	3	250	
(%)	(36.6)	(17.7)	(9.1)	(4.5)	(2.6)	(0.8)	(71.4)	
HPV 6 + 11 [†]	85	50	23	15	7	4	184	52.6 (47.3–57.8)
HPV 16	9	11	7	4	4	2	37	10.6 (7.8–14.3)
HPV 18	4	5	6	5	2	2	24	6.9 (4.6–10.0)
HPV 31	2	4	3	1	4	0	14	4.0 (2.4–6.6)
HPV 33	1	2	2	2	2	1	10	2.9 (1.5–5.2)
HPV 35	0	2	4	1	2	0	9	2.6 (1.3–4.9)
HPV 39	2	5	12	5	5	0	29	8.3 (5.8–11.7)
HPV 45	2	3	0	1	1	0	7	2.0 (1.0–4.0)
HPV 51	1	9	7	5	5	3	30	8.6 (6.1–12)
HPV 52	8	10	7	11	3	3	42	12.0 (9.0–15.8)
HPV 56	3	2	3	3	2	1	14	4.0 (2.4–6.6)
HPV 58	5	10	7	5	6	2	35	10.0 (7.3–13.6)
HPV 59	2	11	5	6	1	0	25	7.1 (4.9–10.4)
HPV 68	3	2	10	0	1	0	16	4.6 (2.8–7.3)

[†]Due to the methodological limitations, it is not possible to differentiate between single and double infection with genotypes 6 and 11.
HPV: Human papillomavirus.

on HPV genotype-specific prevalence in patients with genital warts in order to encourage the implementation of an HPV vaccine program in Beijing, China.

Materials & methods

Patients & sample collection

This was a retrospective study of 350 women diagnosed with genital warts who were treated at the Sexually Transmitted Disease Clinic in Beijing, China between September 2014 and August 2016. Genital epithelial cells and condyloma tissues were scraped and transferred using a liquid-based media for further analysis. The patients' confidentiality was protected by hiding their identities. Informed consent was obtained from all the patients prior to their enrollment in the study. The study design adhered to the principles of the Helsinki Declaration and was approved by the ethics committees of our hospital.

HPV subtype genotyping

DNA from the genital epithelial cells and warts tissues was prepared according to a previous publication [14]. A Chinese State Food and Drug Administration-approved HPV genotyping assay (Liferiver, Shanghai, China) was used to detect HPV genotypes by real-time PCR. This test can individually detect 13 high-risk HPV subtypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68), and the combined low-risk subtypes (6 + 11) [14].

Statistical analysis

The distribution of HPV subtypes was expressed as the percentage of HPV-positive specimens among all cases of genital warts with 95% CI using MetaAnalyst Beta 3.0 software. The association between HPV subtype and patient age was assessed by the χ^2 test performed with SAS9.3 software. A p value of ≤ 0.05 was considered statistically significant.

Results

Characterization of HPV genotypes in women with genital warts

Among 350 cases of genital warts, 13 types of high-risk HPV DNA and 6 + 11 types were detected in 250 of them, for an overall prevalence of 71.4% (Table 1). In the HPV-positive patients, the single HPV genotype infection was 36.6%, and the multiple HPV genotypes infection was 35.0%. Overall, HPV6+11 were found in 52.6% (184/350) of cases. Of the 13 most common high-risk HPV genotypes, HPV52 was detected in 12.0% (42/350), HPV16 in 10.6% (37/350) and HPV58 in 10.0% (35/350) of genital warts cases.

Table 2. High-risk human papillomavirus genotypes distribution among human papillomavirus 6 + 11 positive/negative cases.

Genotype	HPV 6 + 11 positive (n = 184)		HPV6+11 negative (n = 166)	
	No.	%	No.	%
HPV 16	18	9.8 (6.3–15.0)	19	11.4 (7.4–17.2)
HPV18	15	8.1 (5.0–13.1)	9	5.4 (2.8–10.1)
HPV31	9	4.9 (2.6–9.1)	5	3.0 (1.3–7.0)
HPV33	8	4.3 (2.2–8.5)	2	1.2 (0.3–4.7)
HPV35	7	3.8 (1.8–7.8)	2	1.2 (0.3–4.7)
HPV 39	15	8.2 (5.0–13.1)	14	8.4 (5.1–13.7)
HPV45	3	1.6 (0.5–4.9)	2	1.2 (0.3–4.7)
HPV51	27	14.6 (10.3–20.6)	3	1.8 (0.6–5.5)
HPV52	28	15.2 (10.7–21.2)	14	8.4 (5.1–13.7)
HPV56	8	4.3 (2.2–8.5)	6	3.6 (1.6–7.8)
HPV58	25	13.6 (9.3–19.3)	10	6.0 (3.3–10.8)
HPV59	18	9.8 (6.3–15.0)	7	4.2 (2.0–8.6)
HPV68	5	2.7 (1.1–6.4)	11	6.6 (3.7–11.6)

HPV: Human papillomavirus.

Table 3. Low-risk and high-risk infection according to age and correlation between infection and age.

Genotype	<20	21–25	26–30	31–35	36–40	>41	Total
6 + 11	4	22	23	18	6	12	85
6 + 11/HR [†]	6	33	36	13	3	8	99
HR [‡]	2	25	25	6	4	4	66
Others [§]	2	29	30	22	9	8	100
Total	14 (4.0%)	109 (31.1%)	114 (32.6%)	59 (16.9%)	22 (6.3%)	32 (9.1%)	350 (100%)

 χ^2 test for different between ages ($\chi^2 = 18.20$, $p = 0.25$.)[†]6 + 11/HR: 6 + 11 co-infected with HR HPV.[‡]HR: HR HPV infected only.[§]Others: undetected HPV DNA.

HR: High risk; HPV: Human papillomavirus.

Among the 250 detected HPV-positive cases, 127 (50.8%) were infections of a single HPV subtype, including HPV6+11 (n = 85), HPV16 (n = 9) and HPV52 (n = 8) (Table 1). For the remaining HPV-positive cases, 62/250 (24.8%) were infected with two HPV subtypes, 32/250 (12.8%) contained three HPV subtypes, 16/250 (6.4%) had four subtypes, 9/250 (3.6%) had five subtypes and six HPV subtypes were detected in 3/250 (1.2%) cases.

Table 2 shows the prevalence of associated high-risk infections in HPV6+11 positive and negative cases. The three most common high-risk subtypes co-infected with HPV6+11 were HPV52 (15.2%; 95% CI: 10.7–21.2%), HPV51 (14.6%; 95% CI: 10.3–20.6%) and HPV58 (13.6%; 95% CI: 9.3–19.3%). In contrast, HPV16 (11.4%; 95% CI: 7.4–17.2%), HPV39 (8.4%; 95% CI: 5.1–13.7%) and HPV52 (8.4%; 95% CI: 5.1–13.7%) were most common in HPV6+11-negative genital warts cases.

Prevalence of HPV according to age groups

The ages of the 350 patients with genital warts ranged from 16 to 79 years old. Among these, the vast majority (80.9%) of patients were between 21 and 35 years of age. The prevalence of infection with the various HPV subtypes according to patient age is presented in Table 3. No significant differences were observed.

Discussion

This study provides an assessment of the prevalence of HPV6+11 and 13 high-risk HPV genotypes isolated from female patients with genital warts in Beijing. The overall prevalence of HPV infection in genital warts was 71.4%, which is lower than the reported overall HPV prevalence (84.2–88.7%) in China [12]. It is not surprising that not all the HPV subtypes were detected in this study. The results show that the most common infection is from the

low-risk HPV6+11, which was detected in approximately half of the genital warts cases tested. According to the study by Chang *et al.* [11], HPV6+11 prevalence among individuals with genital warts is 65.2% in central areas of China. Other reports show wide variability in infection rates of HPV6 or 11, ranging from 53.6 to 96.4% [12,15,16]. Thus, confirmatory studies are needed to more firmly establish the prevalence of HPV6+11 in different geographic regions in China.

The second most common HPV subtype detected was HPV52, which was found in 12.0% of cases. HPV16 was detected in 10.6% cases of genital warts cases in the present study, which is in agreement with the overall prevalence of HPV16 in China (10.4%) reported by Chang *et al.* [11]. The prevalence of HPV58 was similar, detected in 10.0%. This is the first report detailing the prevalence of HPV52 and HPV58 in genital warts. Furthermore, the fact that the prevalence of HPV16 and HPV58 were common in this study is valuable important, as these subtypes are associated with cervical cancer in Chinese women [17–19].

The results of this study indicate that 35.0% of women with genital herpes have multiple HPV-subtype infections, a prevalence that is higher than that reported by Chang *et al.* [11]. However, this rate falls within the range reported from studies in other countries, where the multiple infection rates in genital warts is 28.3–68.3% [17,20,21]. The results of the present study show that the proportion of cases with HPV6+11 co-infected with HPV 52, 51 or 58 was higher than those co-infected with HPV16, suggesting that the vaccine development for Beijing patients should focus on the combination of these subtypes among women with genital warts. Based on our data, the high-risk HPVs are also highly prevalent in the population with genital warts, this result may be associated with the spread of HPV infection. HPV vaccination program in China may reduce the burden of disease associated with genital warts and achieve performance as good as in western countries. However, based on the results of our study and others, there is a variation of HPV prevalence by geographic regions. Therefore, the effectiveness of a vaccine in reduction of disease burden may differ by population, and the vaccine concluding more HPV subtypes is needed in Beijing, such as 52, 51, 58, 33 and so on.

A cross-sectional study of 18,498 sexually active women 15–74 years of age from the general population of 15 areas in four continents showed that HPV prevalence peaks at approximately 25–35 years of age [22]. Indeed, patients in the present study were generally within this age range, with roughly a third between 21 and 25 years of age and another third between 26 and 30 years. Moreover, a prophylactic quadrivalent HPV6/11/16/18 vaccine has shown high efficacy, safety and tolerability in young Asia-Pacific women [23], though whether this is a cost-effective way for reducing HPV-associated genital warts needs further study. A nonavalent vaccine, which additionally targets HPV31/33/45/52/58, is currently undergoing Phase III clinical trials with the goal of establishing 15–30% higher anticancer effects [24]. This vaccine may be a good candidate for trials in Beijing, as it incorporates the most common HPV subtypes identified in this study.

The present study was performed with 350 patients with women diagnosed with genital warts between 2014 and 2016, which would identify the most common HPV subtypes among women with genital warts in this region. However, there are still some limitations. First, this study was limited to patients from only one hospital in the Beijing area, and more samples need to be detected in this area. Second, a Chinese State Food and Drug Administration-approved HPV genotyping assay (Liferiver, Shanghai, China) was used to detect HPV genotypes by real-time PCR. This test only can individually detect 13 high-risk HPV subtypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68), and the combined low-risk subtypes (6 + 11), and the other HPV subtypes cannot be detected.

Conclusion

In conclusion, the study has reported on the first study of HPV genotype distributions in genital warts patients in Beijing, China. The data suggested women with genital warts in Beijing have more multiple HPV-subtype infections. These results provide a basis for future studies evaluating the vaccines that can be most effective for this population.

Future perspective

About 30–40 HPV types regularly or sporadically infect the mucosal surfaces of the anogenital tract. Although current opinion believe that the 90% genital warts are caused by two low-risk HPV subtypes 6 and 11 [25], the real situation regarding the prevalence and genotype distribution of low-risk HPV in populations with genital warts is rarely reported. There is variation of HPV prevalence by geographic regions, sexes and age groups in China, and the distribution of women HPV subtypes in urban areas is different in rural areas [11]. HPV vaccines are sub-unit

vaccines consisting of virus-like particles) made of one protein – the major HPV coat or capsid protein L1. Gardasil 9 is a nine-valent (nHPV) 6, 11, 16, 18, 31, 33, 45, 52, 58 virus-like particle vaccines from Merck Sharp & Dohme licensed by the US FDA in December 2014 for use in 9–26 year old females and 9–15 year old boys. To achieve optimal vaccine effectiveness immunization ideally should be completed before the start of sexual activity. HPV vaccination programs have now been introduced in many countries. The study about the distribution of HPV subtypes could play an important role in genital warts prevention.

Executive summary

- Genital warts are one of the most common sexually transmitted diseases among young women worldwide that are caused in part by low-risk human papillomavirus (HPV) infection.
- However, the distribution of HPV genotypes varies by geographic location, and more data are needed regarding the prevalence of genotypes in the Beijing region of China.

Methods

- This was a retrospective study of 350 women diagnosed with genital warts who were treated at the Sexually Transmitted Disease Clinic in Beijing, China between September 2014 and August 2016.
- DNA from the genital epithelial cells and warts tissues was prepared, and HPV genotyping assay (Liferiver, Shanghai, China) was used to detect HPV genotypes by real-time PCR. This test can individually detect 13 high-risk HPV subtypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68), and the combined low-risk subtypes (6 + 11).
- The distribution of HPV subtypes was expressed as the percentage of HPV-positive specimens among all cases of genital warts with 95% CIs using MetaAnalyst Beta 3.0 software. The association between HPV subtype and patient age was assessed by the χ^2 test performed with SAS9.3 software. A p-value of ≤ 0.05 was considered statistically significant.

Results

- Among 350 cases of genital warts, 13 types of high-risk HPV DNA and 6 + 11 types were detected in 250 of them, for an overall prevalence of 71.4%.
- The results show that 52.6% (184/350) of cases were positive for HPV6+11. The three most common high-risk HPVs detected were HPV52, HPV16 and HPV58, detected in 12.0% (42/350), 10.6% (37/350) and 10.0% (35/350) of all cases, respectively.
- Of the 250 HPV-positive cases, 49.2% (123/250) were infected by multiple HPV subtypes. Cases infected with HPV6+11 were most commonly co-infected with HPV51, HPV52 and HPV58, whereas HPV16, HPV39 and HPV52 were more common among HPV6+11-negative cases.

Conclusion

- These results indicate that vaccines targeting HPV subtypes 6, 11, 16, 52 and 58 would have the greatest impact among sexually active women living in Beijing.

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