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Human papillomavirus genotyping among patients with genital warts attending a sexual health clinic in Xi'an, China

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ABSTRACT

Objectives: Research on HPV types in genital warts was neglected in Xi'an, China. The objective of this study was to characterize the prevalence and distribution of HPV types in this district.

Methods: In 2014~2017, 23 types of HPV were detected in the specimens of genital warts by automatic nucleic acid hybridization system.

Results: Of the 879 cases of genital warts, the detectable rates of low-risk, of high-risk and of any HPV type(s) were 45.4% (399/879), 34.5% (303/879) and 57.8% (508/879), respectively. The detectable rate of low-risk HPV type(s) (45.4%) was significantly higher than that of high-risk HPV type(s) (34.5%) ($\chi 2=21.85$, $P<0.01$). Of the 512 men, 268 were infected by low-risk (52.3%), 166 by high-risk (32.4%) and 308 by any HPV type(s) (60.2%). Of the 367 women, 131 were infected by low-risk (35.7%), 137 by high-risk (37.3%) and 200 by any HPV type(s) (54.5%). The detectable rate of low-risk HPV type(s) for men (52.3%) was significantly higher than that for women (35.7%) ($\chi 2=23.90$, $P<0.01$). The detectable rates of low-risk, high-risk and any HPV type(s) peaked at the age of 15~19 and 55~59. The detectable rate of single HPV type was 26.1% (229/879), of 2 HPV types was 17.5% (154/879) and of $\geqslant$3 HPV types was 14.2% (125/879), respectively. HPV 6 (24.9%) and 11 (17.9%) were the 2 most prevalent low-risk HPV types, while HPV 52 (9.9%) and 16 (7.3%) were the 2 most prevalent high-risk HPV types.

Conclusion: Our data suggests that current vaccines covering HPV types 6, 11, 16, and 18 are not best vaccines in Xi'an, China and region-specific vaccines covering HPV 6, 11, 52 and 16 are needed.

Strengths and limitations of this study

- This is the first study on HPV type distribution among patients with genital warts in Xi'an, China.
- Results of studies showed that the most prevalent HPV types were HPV 6, 11, 52 and 16 in Xi'an, China.
- Current vaccines covering HPV types 6, 11, 16, and 18 are not best vaccines in Xi'an, China.

- Region-specific vaccines covering HPV 6, 11, 52 and 16 are needed in Xi'an, China.

INTRODUCTION

Genital warts are symptoms of a contagious sexually transmitted disease caused by certain types of human papillomavirus (HPV). Previous studies have shown that infection with HPV 6 or 11 was associated with the majority of cases of genital warts.[1] Therefore, some HPV vaccines that can target HPV 6 and 11 have shown efficacy to prevent genital warts. The reduction of genital warts incidence has been reported in many countries where prophylactic HPV vaccination programs have been adopted.[2] China Food and Drug Administration approved Human Papillomavirus Absorbed Vaccine (Cervarix, GlaxoSmithKline) on July 12, 2016 in order to prevent cervical cancer and other HPV-related diseases more effectively.[3] However, the distribution of HPV types varies among different countries or districts.[4 5] Therefore, geographically specific HPV vaccines are required to be developed according to local HPV type prevalence. Our clinic, Shaanxi Provincial Institute for Skin Disease and STD Control, is a large sexual health clinic in Xi'an, China. Data of HPV type prevalence from our clinic can well represent that of Xi'an. In 2015, 1039 genital warts cases were reported in Xi'an, and 602 cases were diagnosed in our clinic. In present study, we investigated prevalence and distribution of HPV types among patients with genital warts in our clinic. Information provided in this study will facilitate geographically specific HPV vaccination in Xi'an to prevent genital warts and other HPV-related diseases.

MATERIALS AND METHODS

Participants and specimen collection

The present study was conducted from September 2014 to April 2017. Clients who visited to Shaanxi Provincial Institute for Skin Disease and STD Control for genital warts screening were recruited in the study and signed informed consent before enrollment. They were screened for genital warts according to the diagnostic criteria from United States Centers for Disease Control.[6] The diagnosis were most often made visually as genital warts characteristically rose above the skin surface due to enlargement of dermal papillae and parakeratosis, while an acetic acid solution was used to identify smaller warts which were difficult to diagnose by sight. Genital warts were detected in a total of 879 clients. These 879 patients were selected as participants in this study and further underwent HPV genotyping. They were aged from 15 to 65 years and none of them received any HPV vaccination before enrollment. Specimens for HPV genotyping were collected from lesions of warts by using a cell brush to collect shedding cells that might be infected with viruses and stored at -80℃ before testing.

HPV genotyping

23 types of HPV were detected from the stored specimens. An automatic nucleic acid hybridization system manufactured by Yaneng Bioscience (Shenzhen) Co.,Ltd were employed. 23 types of human papillomavirus genotyping detection kit manufactured by Yaneng Bioscience (Shenzhen) Co.,Ltd. were used. All procedures were carried out following the manufacturer's instructions. HPV genotyping techniques used both DNA amplification and hybridization to simultaneously identify 23 HPV genotypes, including 5 low-risk HPV types (HPV 6, 11, 42, 43, and 81), and 18 high-risk HPV types (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 82 and 83). Quality control was implemented during HPV DNA detection.

Ethical approval

Approval for collection of specimens from patients with genital warts and HPV testing of the stored specimens was obtained from Ethics Committee of Shaanxi Provincial Institute for Skin Disease and STD Control (approval number: SXEDC2017-001).

Statistical Analysis

All data were analyzed using SPSS software (version 16.0). R×C chi-square test was used for comparing differences among groups, with $\alpha \leqslant 0.05$ considered statistically significant. A correlation coefficient was calculated to quantify statistical relationship between 2 values, with $\alpha \leqslant 0.05$ considered significant.

Patient and public involvement

Specimens of patients were collected from lesions of warts. There was no further involvement in the implementation of this study by patients or the public.

RESULTS

Prevalence of low-risk and high-risk HPV types in patients with genital warts among different age groups

Specimens of 879 patients including 512 men and 367 women with genital warts were tested for 5 low-risk and 18 high-risk HPV types. As showed in Table 1, of the 512 men, 268 were infected by low-risk (52.3%), 166 by high-risk (32.4%) and 308 by any HPV type(s) (60.2%). Of the 367 women, 131 were infected by low-risk (35.7%), 137 by high-risk (37.3%) and 200 by any HPV type(s) (54.5%). The detectable rate of low-risk HPV type(s) for men (52.3%) was significantly higher than that for women (35.7%) ($\chi2=23.90$, $P<0.01$), while the detectable rate of high-risk HPV type(s) for men (32.4%) was not significantly different with that for women (37.3%) ($\chi2=2.28$, $P=0.13$). There was no significant difference between the detectable rate of any HPV type(s) for men (60.2%) and that for women (54.5%) ($\chi2=2.81$, $P=0.09$). The overall detectable rate of low-risk HPV type(s), of high-risk HPV type(s), and of any HPV type(s) were 45.4%, 34.5% and 57.8%, respectively. The overall detectable rate of low-risk HPV type(s) (45.4%) was significantly higher than that of high-risk HPV type(s) (34.5%) ($\chi2=21.85$, $P<0.01$) . As showed in Table 1, we divided patients with genital warts into 10 age groups, the detectable rates of low-risk, high-risk and any HPV type(s) peaked at the age of 15~19 and 55~59.

Table 1. Distribution of genital warts cases and prevalence of low-risk and high-risk HPV among age groups in Xi'an

Age	Cases (n, %)			Low-risk type (n, %)			High-risk type (n, %)			Any type (n, %)		
(years)	Male	Female	Total	Male	Female	Total	Male	Female	Total	Male	Female	Total
15~	6(1.2)	11(3.0)	17(1.9)	4(66.7)	6(54.5)	10 (58.8)	2(33.3)	4(36.4)	6 (35.3)	4(66.7)	7(63.6)	11 (64.7)
20~	73(14.3)	79(21.5)	152 (17.3)	42(57.5)	24(30.4)	66 (43.4)	22(30.1)	29(36.7)	51 (33.6)	46(63.0)	38(48.1)	84 (55.3)
25~	130 (25.4)	102 (27.8)	232 (26.4)	63(48.5)	39(38.2)	102 (44.0)	38(29.2)	43(42.2)	81 (34.9)	69(53.1)	58(56.9)	127 (54.7)

30~	90 (17.6)	57(15.5)	147 (16.7)	42(46.7)	20(35.1)	62 (42.2)	23(25.6)	22(38.6)	45 (30.6)	47(52.2)	30(52.6)	77 (52.4)
35~	58(11.3)	43(11.7)	101 (11.5)	31(53.4)	15(34.9)	46 (45.5)	24(41.4)	15(34.9)	39 (38.6)	40(69.0)	23(53.5)	63 (62.4)
40~	52(10.2)	36(9.8)	88 (10.0)	20(38.5)	13(36.1)	33 (37.5)	16(30.8)	12(33.3)	28 (31.8)	27(51.9)	21(58.3)	48 (54.5)
45~	52(10.2)	21(5.7)	73 (8.3)	29(55.8)	8(38.1)	37(50.7)	20(38.5)	5(23.8)	25(34.2)	36(69.2)	12(57.1)	48(65.8)
50~	30(5.9)	13(3.5)	43 (4.9)	20 (66.7)	5 (38.5)	25 (58.1)	12(40.0)	6(46.2)	18 (41.9)	22(73.3)	9(69.2)	31(72.1)
55~	12(2.3)	3(0.8)	15 (1.7)	10 (83.3)	1 (33.3)	11 (73.3)	6 (50.0)	1 (33.3)	7 (46.7)	10(83.3)	2(66.7)	12(80.0)
60~	9(1.8)	2(0.5)	11 (1.2)	7 (77.8)	0 (0.0)	7 (63.6)	3(33.3)	0(0.0)	3(27.3)	7(77.8)	0(0.0)	7(63.6)
Total	512(100.0)	367(100.0)	879(100.0)	268 (52.3)	131(35.7)	399(45.4)	166(32.4)	137(37.3)	303(34.5)	308(60.2)	200(54.5)	508(57.8)

Prevalence of each specific HPV type in patients with genital warts

As showed in Table 2, the detectable rate of HPV 6 (24.9%), HPV 11 (17.9%), HPV 52 (9.9%), and HPV 16 (7.3%) ranked top 4 among the 23 HPV types. The detectable rate of HPV 6 (24.9%) was significantly higher than that of HPV 11 (17.9%) ($\chi2=13.00$, $P<0.01$), and the detectable rate of HPV 11 (17.9%) was significantly higher than those of the remaining low-risk HPV types, such as HPV 42, 43, and 81 (5.2%~5.3%) ($\chi2\geqslant67.10$, $P<0.01$). So, HPV 6 and 11 were the 2 most commonly prevalent low-risk HPV types among 5 low-risk HPV types. The detectable rate of HPV 6/11 were 38.6%. Among the 18 high-risk HPV types, the detectable rate of HPV 52 (9.9%) was significantly higher than that of the other 17 high-risk HPV types (0.1%~7.3%) ($\chi2\geqslant3.83$, $P\leqslant0.05$). The detectable rate of HPV 16 (7.3%) was not significantly higher than that of HPV 58 (5.2%) ($\chi2=3.14$, $P=0.08$), but the detectable rate of HPV 16 (7.3%) was significantly higher than that of the remaining 15 high-risk types (HPV 68, 51, 56, 53, 18, 66, 59, 39, 33, 31, 73, 35, 83, 45 and 82 (0.1% to 4.4%) ($\chi2\geqslant6.45$, $P\leqslant0.01$). So, HPV 52 and 16 were the 2 most commonly prevalent high-risk HPV types. Although HPV 58(5.2%) ranked third among the 18 high-risk HPV types, it was not yet confirmed that HPV58 is a commonly prevalent high-risk HPV type because the rates of HPV 58, 68, 51, 56, 53, 18, 66 and 59, ranged from 3.4% to 5.2%, were not significantly different ($\chi2\leqslant3.52$, $P\geqslant0.05$). We could exclude HPV 39, 33, 31, 73, 35, 83, 45 and 82 from the commonly prevalent high-risk HPV types, because the detectable rates of these types (0.1%~2.2%) were significantly lower than those of other 8 HPV types, such as HPV 52, 16, 58, 68, 51, 56, 53 and 18 (4.0%~9.9%) ($\chi2\geqslant4.91$, $P\leqslant0.03$). So, we can confirmed that HPV 6 (24.9%), HPV 11 (17.9%), HPV 52 (9.9%) and 16 (7.3%) the 4 most prevalent HPV types in genital warts in Xi'an, China.

Table 2. Distribution of HPV types in patients with genital warts in Xi'an [n(male)=512; n(female)=367; n(total)=879]

Type	Male (n,%)	Female (n,%)	Total (n,%)	The most prevalent
Low-risk				

6	150 (29.3)	69 (18.8)	219 (24.9)	yes
11	110 (21.5)	47 (12.8)	157 (17.9)	yes
42	27 (5.3)	20 (5.4)	47 (5.3)	no
43	33 (6.4)	14 (3.8)	47 (5.3)	no
81	30 (5.9)	16 (4.4)	46 (5.2)	no
High-risk				
52	54 (10.5)	33 (9.0)	87 (9.9)	yes
16	33 (6.4)	31 (8.4)	64 (7.3)	yes
58	24 (4.7)	22 (6.0)	46 (5.2)	not confirmed
68	30 (5.9)	9 (2.5)	39 (4.4)	not confirmed
51	24(4.7)	14(3.8)	38 (4.3)	not confirmed
56	22(4.3)	16(4.4)	38 (4.3)	not confirmed
53	18(3.5)	19(5.2)	37 (4.2)	not confirmed
18	22(4.3)	13(3.5)	35 (4.0)	not confirmed
66	19(3.7)	12(3.3)	31 (3.5)	not confirmed
59	17(3.3)	13(3.5)	30 (3.4)	not confirmed
39	10 (2.0)	9 (2.5)	19 (2.2)	no
33	5(1.0)	11(3.0)	16 (1.8)	no
31	8(1.6)	6(1.6)	14 (1.6)	no
73	9(1.8)	4(1.1)	13 (1.5)	no
35	7(1.4)	5(1.4)	12 (1.4)	no
83	4(0.9)	3(0.8)	7 (0.8)	no
45	5(1.0)	1(0.3)	6 (0.7)	no
82	1 (0.2)	0 (0.0)	1 (0.1)	no

Any	308 (60.2)	200 (54.5)	508 (57.8)

Single HPV type infections or multiple HPV type co-infections in patients with genital warts

As showed in Table 3, the detectable rate of single HPV type was 26.1% (229/879), while the detectable rate of co-infections of 2 and ⩾3 HPV types were 17.5% (154/879) and 14.2% (125/879), respectively. The detectable rate of single HPV type infections (26.1%) were significantly higher than that of 2 HPV type co-infections (17.5%) ($\chi2$=18.77, P<0.01) and that of ⩾3 HPV type co-infections (14.2%) ($\chi2$=38.26, P<0.01). The number of infecting HPV type ranged from 1 to 13. The number of infecting HPV types had a negative correlation with the detectable rate (r=-0.7258, P=0.05), but there was no correlation between the number of infecting HPV type and age (r=-0.0086，P=0.50).

Table 3. Prevalence of single infections and co-infections of HPV types in genital warts in Xi'an (n=879)

Type number	Infection (n,%)	Years of age ($\bar{X}$, S)
1	229 (26.1)	34.9 (10.8)
2	154 (17.5)	32.5 (10.5)
3	65 (7.4)	34.2 (10.8)
4	20 (2.3)	33.5 (10.2)
5	20 (2.3)	30.5 (6.8)
6	10 (1.1)	31.3 (14.5)
7	8 (0.9)	32.0 (13.0)
8	1 (0.1)	34.0 (0.0)
13	1 (0.1)	22.0 (0.0)
Any	508 (57.8)	33.1 (10.0)

DISCUSSION

HPV, as one of the seven notorious carcinogenic viruses, has been studied worldwide. Genital warts are the most common clinical manifestation of nononcogenic HPV infection. Although non-life threatening, genital warts carry a substantial psychosocial and economic burden. Patients of genital warts are disturbed by the shame and embarrassment related to diagnosis, as well as the inconvenience and discomfort of treatment and the fear of recurrence, transmission, and the possible threat of cancer. Co-infections with multiple HPV types are possible and may combine both low-risk and high-risk types, even in cases of genital warts.[7] Recently, significant progresses in control and prevention of HPV infections have been made, and prophylactic HPV vaccines have been used successfully to prevent HPV-related cancers and genital warts.[8] However, genotypes of HPV are over 100 and the distribution of these genotypes differs regionally.[9 10] Although vaccines can protect population in some places, but they can not protect population in other places because of the regional difference of HPV genotype distribution. Thus, information of HPV genotype distribution is of importance for vaccine development in a given area.

Actually, HPV infection rates with genital warts varies among reports. Previous multinational study on HPV in men from Tampa, Florida, Cuernavaca, Mexico, and Sao Paulo, Brazil reported that the detectable rates of low-risk and high-risk HPV genotypes were 73.2% and 15.6% of external genital lesions in men, respectively.[11] A study on HPV in women from Australia found that HPV DNA was detected in 90.8% of genital warts, with HPV 6 and HPV 11 detected in 86.0% of genital warts and high-risk HPV types detected in 31.0% of genital warts.[12] Another study on genital warts in men in Hong Kong reported that HPV 6/11 was found in 63.1% and HPV 16/18 was found in 9.2% of genital warts.[13] Our study showed that the detectable rates of low-risk and high-risk HPV types were 45.4% and 34.5% in genital warts patients in Xi'an, China. Although HPV infection rates with genital warts varies among reports, the above mentioned reports and our study indicate that low-risk HPV types are major causative agents of genital warts, but the prevalence of high-risk HPV types in population with genital warts is an issue that cannot be ignored. Since genital warts is a sexually transmitted disease, high-risk HPV types in genital warts can act as reservoirs of cancer-related HPV types to threat local population.

The most prevalent genotypes in a given area is one of the areas of the most concern. One study form USA found that HPV 6 (43.8%), HPV 11 (10.7%), and HPV 16 (9.8%) were the genotypes most commonly detected in genital warts in men.[14] Another study from Bogota, Colombia found that HPV 6 was by far the most common type in both women (62%) and men (56%), followed by HPV 11 (20%). HPV16 ranked third in prevalence, with 16% of patients tested positive in genital warts.[15] A study from seven geographical regions of China reported that the most prevalent genotypes were HPV 6 (41.3%), HPV 11 (37.6%) and HPV 16 (10.4%) in genital warts.[16] Our study found that HPV 6 (24.9%), HPV 11 (17.9%), HPV 52 (9.9%) and 16 (7.3%) were the most prevalent HPV types in Xi'an, China. Our data agreed with the report from Guangdong, China that the most prevalent genotypes were HPV 6 (42.2%), HPV 11 (39.3%), HPV 52 (7.7%), and HPV 16 (7.56%) in patients with genital warts.[17] The above mentioned reports and our study indicated that HPV 6, 11, and 16 were the most prevalent genotypes in genital warts in many areas of the world. Additionally, HPV 52 was one of the most prevalent genotypes in genital warts in Xi'an and also in Guangdong, China. Whether HPV 52 is one of the most prevalent genotypes in other areas of the world needs to be confirmed. The relatively low prevalence of HPV 18 in genital warts is interesting, as it indicates that current available quadrivalent vaccines covering HPV 6, 11, 16, and 18 may not be a best candidate for prevention of genital warts in Xi'an and region-specific vaccines covering HPV 6, 11, 52 and 16 are needed.

The gender differences of HPV type prevalence varied among reports. In our study, the detectable rate of low-risk HPV types in men was significantly higher than that in women (52.3% vs 35.7%, $P<0.01$), while the detectable rate of high-risk HPV in men was not significantly different with that for women (32.4% vs 37.3%, $P=0.13$). A study from South Africa agreed with our study in that the prevalence of non-carcinogenic HPV in men

(33.2%) was higher than that in women (14.0%), but the prevalence of carcinogenic HPV was similar in men (22.4%) and women (22.7%).[18] On the contrary, a study form Liuzhou, China found that the prevalence of nononcogenic HPV type was similar (1.4% vs 1.2%, P=0.6832), whereas the prevalence of oncogenic HPV type was higher in women than in men (18.7% vs 9.4%, P<0.001).[19] At present, it is impossible to summarize the gender differences in HPV type prevalence in genital warts. Widdice et al. suggested that, compared with men, women may have a higher incidence rate or lower clearance rate of HPV infections because of the different physiological structures between genders.[20] Data from Guangdong, China suggested that infected men constituted an important viral reservoir, contributing to transmission of high-risk HPV to women and maintenance of infections, but high-risk HPV infections might be less likely to persist in men than in women.[21] Further prospective studies should be conducted to illustrate the differences in HPV natural history in both sexes.

It also showed in a report that the rate of HPV infections manifested two age-peaks at ≤25 years and >65 years of age. Moreover, most HPV infections among the young patients were transient, and only 5-10% of the infections would become persistent.[22] A recent report from Guiyang, China also observed two age-peaks of HPV infections, one at the age of ≤20 and the other at the age of 55~59.[23] We found the similar results that low-risk, high-risk and any type HPV detectable rates had 2 age-peaks at the age of 15~19 and 55~59. The age-peaks of HPV infection rate can be explained as patients of these 2 age groups are either too young or too old to have a stronger immunity particularly for protection against HPV infection. Peng et al. suggested that most patients at the first peak got low-risk HPV and caused genital warts, whereas most patients with cervical lesions, including cervical cancer (CC) and cervical intraepithelial neoplasia (CIN), contributed to the second peak at an older age.[23]

Finally, we found that co-infections of HPV types were a common phenomenon in genital warts, as the detectable rates of 1, 2 and ≥3 HPV types were 26.1%, 17.5% and 14.2%, respectively in our study. The phenomenon of multiple HPV type infections was also found before. For example, Brown et al. used probes for both low- and high-risk HPV types, and they found that 16.3% of genital warts from healthy patients and 52.3% of genital warts form patients with altered cell-mediated immunity, respectively, were positive for both probes.[24] In a retrospective analysis of specimens of women from USA, the results showed that 4.6% of entire sample and 19.0% of HPV-positive sample were positive for 2 or more HPV types.[25] Data from Italy showed that multiple HPV infections occurred by chance and there was no evidence to show that specific HPV types had the tendency to be found more or less often than others in co-infections.[26] A interesting finding about multiple HPV type infections in genital warts in our study was that the number of infecting HPV types did not accumulate with age increasing, because statistical analysis showed that there was no correlation between infecting HPV type numbers and age.

In conclusion, the present study characterized features of epidemiology of HPV types in patients with genital warts in Xi'an, China. Our data will provide an important foundation for prevention, diagnosis and treatment of HPV infections and suggest that development of vaccines for prevention and treatment of genital warts and other HPV-related diseases in this area should target HPV types 6, 11, 52 and 16, but current quadrivalent vaccines covering HPV types 6, 11, 16, and 18 are not best vaccines in Xi'an, China.

The study was limited by the relatively small sample size. All participants recruited for the study were clients who visited our clinic for genital warts screening and therefore not a good representative of the general population.

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Contributors The study was designed by CSZ. WHM collected specimens. YFW, HSZ, and JJM performed clinical diagnosis of genital warts. CSZ and YFW analyzed data. CSZ interpreted and drafted the paper. All authors approved the final version of the article.

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Prevalence of low-risk and high-risk HPV types in genital warts: a cross-sectional study in Xi'an, China

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Prevalence of low-risk and high-risk HPV types in genital warts: a cross-sectional study in Xi'an, China

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ABSTRACT

Objectives To characterize the prevalence and distribution of HPV types in Xi'an, China.

Design A cross-sectional study.

Setting The study was conducted in Xi'an in northwest China during September 2014 to April 2017.

Participants A total of 912 cases of genital warts were eligible for this study. Among them, 879 cases were recruited in this study.

Results Of the 879 cases of genital warts, the detectable rates of low-risk, high-risk and any HPV type(s) were 45.4% (399/879), 34.5% (303/879) and 57.8% (508/879), respectively. The detectable rate of low-risk HPV type(s) (45.4%) was significantly higher than that of high-risk HPV type(s) (34.5%) ($\chi 2=21.85$, $P<0.01$). Of the 512 men, 268 were infected by low-risk (52.3%), 166 by high-risk (32.4%) and 308 by any HPV type(s) (60.2%). Of the 367 women, 131 were infected by low-risk (35.7%), 137 by high-risk (37.3%) and 200 by any HPV type(s) (54.5%). The detectable rate of low-risk HPV type(s) in men (52.3%) was significantly higher than that in women (35.7%) ($\chi 2=23.90$, $P<0.01$). The detectable rates of low-risk, high-risk and any HPV type(s) peaked at the age of 15~19 and 55~59. The detectable rate of single HPV type was 26.1% (229/879), of 2 HPV types was 17.5% (154/879) and of $\geqslant$3 HPV types was 14.2% (125/879), respectively. HPV 6 (24.9%) and 11 (17.9%) were the 2 most prevalent low-risk HPV types, while HPV 52 (9.9%) and 16 (7.3%) were the 2 most prevalent high-risk HPV types.

Conclusion HPV 6, 11, 52 and 16 are the most common HPVs in genital warts in Xi'an, China. The burden of genital warts can potentially be lowered with increasing usage of vaccination.

Strengths and limitations of this study

- This is the first study evaluating the prevalence and HPV type distribution of genital warts in Xi'an, China.
- Diagnoses of genital warts according to epidemiological history and skin lesions in this study could potentially result in misdiagnosis and missed diagnosis.

- Patients of genital warts who did not seek treatment were not included.
- Patients of genital warts who sought treatment in other clinics in Xi'an were not included.

INTRODUCTION

Genital warts are symptoms of a contagious sexually transmitted disease caused by certain types of human papillomavirus (HPV). Previous studies have shown that infection with HPV 6 or 11 was associated with the majority of cases of genital warts.[1] Therefore, some HPV vaccines that can target HPV 6 and 11 have shown efficacy to prevent genital warts. The reduction of genital warts incidence has been reported in many countries where prophylactic HPV vaccination programs have been adopted.[2] China Food and Drug Administration approved Human Papillomavirus Absorbed Vaccine (Cervarix, GlaxoSmithKline) on July 12, 2016 in order to prevent cervical cancer and other HPV-related diseases more effectively.[3] However, the distribution of HPV types varies among different countries or districts.[4,5] Therefore, geographically specific HPV vaccines are required to be developed according to local HPV type prevalence. Our clinic, Shaanxi Provincial Institute for Skin Disease and STD Control, is a large sexual health clinic in Xi'an, China. Data of HPV type prevalence from our clinic can well represent that of Xi'an. In 2015, 1039 genital warts cases were reported in Xi'an, and 602 cases were diagnosed in our clinic. In present study, we investigated prevalence and distribution of HPV types in genital warts in our clinic. Information provided in this study will facilitate geographically specific HPV vaccination in Xi'an to prevent genital warts and other HPV-related diseases.

METHODS

Study design

The present study was a cross-sectional study during September 2014 to April 2017. Patients who attended the Clinic, Shaanxi Provincial Institute for Skin Diseases and STD, for the treatment of newly diagnosed genital warts were invited to participate. Specimens from lesions of warts were collected and 23 types of HPV were detected.

Inclusion and exclusion criteria

Patients of genital warts were diagnosed according to the diagnostic criteria of genital warts of China.[6] Briefly, they had epidemiological history, such as unsafe sex(es), or sexual partner(s) with a history of genital warts, or multiple sexual partners. Additionally, they had skin lesions. Skin lesions appeared as needles or mung bean-sized papules. Skin lesions could gradually increase to papillary, cockscomb, cauliflower-like masses. Patients who had the above-mentioned epidemiological history and skin lesions were diagnosed as genital warts cases. Acetic acid white test was used in differential diagnosis and positive results confirmed the diagnosis of genital warts when skin lesions were atypical. Newly diagnosed patients of genital warts were eligible for the study. Patients who were not permanent residents of Xi'an City, or who had warts in non genital area, were excluded from the study..

Participants

A total of 912 cases of genital warts were eligible for this study during September 2014 to April 2017. Among them, 33 cases declined to participate. The remaining 879 cases including 512 men and 367 women were recruited in this study. They were between 16 and 28 years old and none of them received any HPV vaccination before enrollment.

Specimen collection

Specimens from lesions of warts were collected by using a cell brush to collect shedding cells that might be infected with viruses and stored at -80□ before testing.

HPV genotyping

HPV testing was the standard of care at our clinic. An automatic nucleic acid hybridization system manufactured by Yaneng Bioscience (Shenzhen) Co.,Ltd was employed. 23 types of human papillomavirus genotyping detection kit manufactured by Yaneng Bioscience (Shenzhen) Co.,Ltd. was used to detect HPV in the specimens. All procedures were carried out following the manufacturer's instructions. HPV genotyping techniques used both DNA amplification and hybridization to simultaneously identify 23 HPV genotypes, including 5 low-risk HPV types (HPV 6, 11, 42, 43, and 81), and 18 high-risk HPV types (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 82 and 83). Quality control was implemented during HPV DNA detection.

Ethical approval

Approval for collection of specimens from patients of genital warts and HPV testing was obtained from Ethics Committee of Shaanxi Provincial Institute for Skin Disease and STD Control (approval number: SXEDC2017-001).

Statistical Analysis

All data were analyzed using SPSS software (version 16.0). R×C chi-square test was used for comparing differences among groups, with $\alpha \leqslant 0.05$ considered statistically significant. A correlation coefficient was calculated to quantify statistical relationship between 2 values, with $\alpha \leqslant 0.05$ considered significant.

Patient and public involvement

Specimens of patients from lesions of warts were collected. There was no further involvement in the implementation of this study by patients or the public.

RESULTS

Prevalence of low-risk and high-risk HPV types in genital warts

Specimens of 879 patients including 512 men and 367 women with genital warts were tested for 5 low-risk and 18 high-risk HPV types. As showed in Table 1, of the 512 men, 268 were infected by low-risk (52.3%), 166 by high-risk (32.4%) and 308 by any HPV type(s) (60.2%). Of the 367 women, 131 were infected by low-risk (35.7%), 137 by high-risk (37.3%) and 200 by any HPV type(s) (54.5%). The detectable rate of low-risk HPV type(s) in men (52.3%) was significantly higher than that in women (35.7%) ($\chi 2=23.90$, $P<0.01$), while the detectable rate of high-risk HPV type(s) in men (32.4%) was not significantly different with that in women (37.3%) ($\chi 2=2.28$, $P=0.13$). There was no significant difference between the detectable rate of any HPV type(s) in men (60.2%) and that in women (54.5%) ($\chi 2=2.81$, $P=0.09$).

The overall detectable rate of low-risk, high-risk, and any HPV type(s) were 45.4%, 34.5% and 57.8%, respectively. The overall detectable rate of low-risk HPV type(s) (45.4%) was significantly higher than that of high-risk HPV type(s) (34.5%) ($\chi 2=21.85$, $P<0.01$) .

Table 1. Prevalence of low-risk and high-risk HPV types in genital warts in Xi'an (n=879)

Gender	Cases (n)	Low-risk type (n,%)	High-risk type (n,%)	Any type (n,%)
Men	512	268 (52.3)	166 (32.4)	308 (60.2)
Women	367	131 (35.7)	137 (37.3)	200 (54.5)
Total	879	399 (45.4)	303 (34.5)	508 (57.8)

Prevalence of low-risk and high-risk HPV among age groups

As showed in Table 2, we divided patients with genital warts into 10 age groups. Of the 879 cases of genital warts, 95.1% came from sexually active age of 20~54 years. The detectable rates of low-risk, high-risk and any HPV type(s) peaked at the age of 15~19 and 55~59.

Table 2. Prevalence of low-risk and high-risk HPV among age groups in Xi'an (n=879)

Age (years)	Cases (n, %)	Any type (n, %)	High-risk type (n, %)	Low-risk type (n, %)
15~	17 (1.9)	11 (64.7)	6 (35.3)	10 (58.8)
20~	152 (17.3)	84 (55.3)	51 (33.6)	66 (43.4)
25~	232 (26.4)	127 (54.7)	81 (34.9)	102 (44.0)
30~	147 (16.7)	77 (52.4)	45 (30.6)	62 (42.2)
35~	101 (11.5)	63 (62.4)	39 (38.6)	46 (45.5)
40~	88 (10.0)	48 (54.5)	28 (31.8)	33 (37.5)
45~	73 (8.3)	48 (65.8)	25 (34.2)	37 (50.7)
50~	43 (4.9)	31 (72.1)	18 (41.9)	25 (58.1)
55~	15 (1.7)	12 (80.0)	7 (46.7)	11 (73.3)
60~	11 (1.2)	7 (63.6)	3 (27.3)	7 (63.6)
Total	879 (100.0)	508 (57.8)	303 (34.5)	399 (45.4)

Prevalence of each specific HPV type in genital warts

As showed in Table 3, the detectable rate of HPV 6 (24.9%), HPV 11 (17.9%), HPV 52 (9.9%), and HPV 16 (7.3%) ranked top 4 among the 23 HPV types. The detectable rate of HPV 6 (24.9%) was significantly higher than that of HPV 11 (17.9%) ($\chi2$=13.00, P<0.01), and the detectable rate of HPV 11 (17.9%) was significantly higher than those of the remaining low-risk HPV types, such as HPV 42, 43, and 81 (5.2%~5.3%) ($\chi2 \geqslant 67.10$, P<0.01). So, HPV 6 and 11 were the 2 most commonly prevalent low-risk HPV types among 5 low-risk HPV types. The detectable rate of HPV 6/11 were 38.6%. Among the 18 high-risk HPV types, the detectable rate of HPV 52 (9.9%) was significantly higher than that of the other 17 high-risk HPV types (0.1%~7.3%) ($\chi2 \geqslant 3.83$, $P \leqslant 0.05$). The detectable rate of HPV 16 (7.3%) was higher than that of HPV 58 (5.2%) ($\chi2$=3.14, P=0.08), and the detectable rate of HPV 16 (7.3%) was significantly higher than that of the remaining 15 high-risk types (HPV 68, 51, 56, 53, 18, 66, 59, 39, 33, 31, 73, 35, 83, 45 and 82 (0.1% to 4.4%) ($\chi2 \geqslant 6.45$, $P \leqslant 0.01$). So, HPV 52 and 16 were the 2 most commonly prevalent high-risk HPV types. Although HPV 58(5.2%) ranked third among the 18 high-risk HPV types, it was not yet confirmed that HPV58 was a commonly prevalent high-risk HPV type because the rates of HPV 58, 68, 51, 56, 53, 18, 66 and 59, ranged from 3.4% to 5.2%, were not significantly different ($\chi2 \leqslant 3.52$, $P \geqslant 0.05$). We could exclude HPV 39, 33, 31, 73, 35, 83, 45 and 82 from the commonly prevalent high-risk HPV types, because the detectable rates of these types (0.1%~2.2%) were significantly lower than those of other 8 HPV types, such as HPV 52, 16, 58, 68, 51, 56, 53 and 18 (4.0%~9.9%) ($\chi2 \geqslant 4.91$, $P \leqslant 0.03$). So, we could confirmed that HPV 6 (24.9%), HPV 11 (17.9%), HPV 52 (9.9%) and 16 (7.3%) were the 4 most prevalent HPV types in genital warts in Xi'an, China.

Table 3. Distribution of HPV types in genital warts in Xi'an [n(male)=512; n(female)=367; n(total)=879]

Type	Male (n,%)	Female (n,%)	Total (n,%)	The most prevalent
Low-risk				
6	150 (29.3)	69 (18.8)	219 (24.9)	yes
11	110 (21.5)	47 (12.8)	157 (17.9)	yes
42	27 (5.3)	20 (5.4)	47 (5.3)	no
43	33 (6.4)	14 (3.8)	47 (5.3)	no
81	30 (5.9)	16 (4.4)	46 (5.2)	no
High-risk				
52	54 (10.5)	33 (9.0)	87 (9.9)	yes
16	33 (6.4)	31 (8.4)	64 (7.3)	yes
58	24 (4.7)	22 (6.0)	46 (5.2)	not confirmed
68	30 (5.9)	9 (2.5)	39 (4.4)	not confirmed
51	24(4.7)	14(3.8)	38 (4.3)	not confirmed

56	22(4.3)	16(4.4)	38 (4.3)	not confirmed
53	18(3.5)	19(5.2)	37 (4.2)	not confirmed
18	22(4.3)	13(3.5)	35 (4.0)	not confirmed
66	19(3.7)	12(3.3)	31 (3.5)	not confirmed
59	17(3.3)	13(3.5)	30 (3.4)	not confirmed
39	10 (2.0)	9 (2.5)	19 (2.2)	no
33	5(1.0)	11(3.0)	16 (1.8)	no
31	8(1.6)	6(1.6)	14 (1.6)	no
73	9(1.8)	4(1.1)	13 (1.5)	no
35	7(1.4)	5(1.4)	12 (1.4)	no
83	4(0.9)	3(0.8)	7 (0.8)	no
45	5(1.0)	1(0.3)	6 (0.7)	no
82	1 (0.2)	0 (0.0)	1 (0.1)	no
Any	308 (60.2)	200 (54.5)	508 (57.8)	

Single HPV type infections or multiple HPV type co-infections in genital warts

As showed in Table 4, the detectable rate of single HPV type was 26.1% (229/879), while the detectable rate of co-infections of 2 and ⩾3 HPV types were 17.5% (154/879) and 14.2% (125/879), respectively. The detectable rate of single HPV type infections (26.1%) were significantly higher than that of 2 HPV type co-infections (17.5%) ($\chi 2=18.77$, $P<0.01$) and that of ⩾3 HPV type co-infections (14.2%) ($\chi 2=38.26$, $P<0.01$). The number of infecting HPV type ranged from 1 to 13. The number of infecting HPV types had a negative correlation with the detectable rate ($r=-0.7258$, $P=0.05$), but there was no correlation between the number of infecting HPV type and age ($r=-0.0086$, $P=0.50$).

Table 4. Prevalence of single infections and co-infections of HPV types in genital warts in Xi'an (n=879)

Type number	Infection (n,%)	Years of age ($\bar{X}$, S)
1	229 (26.1)	34.9 (10.8)

2	154 (17.5)	32.5 (10.5)
3	65 (7.4)	34.2 (10.8)
4	20 (2.3)	33.5 (10.2)
5	20 (2.3)	30.5 (6.8)
6	10 (1.1)	31.3 (14.5)
7	8 (0.9)	32.0 (13.0)
8	1 (0.1)	34.0 (0.0)
13	1 (0.1)	22.0 (0.0)
Any	508 (57.8)	33.1 (10.0)

DISCUSSION

HPV, as one of the seven notorious carcinogenic viruses, has been studied worldwide. Genital warts are the most common clinical manifestation of nononcogenic HPV infection. Although non-life threatening, genital warts carry a substantial psychosocial and economic burden. Patients of genital warts are disturbed by the shame and embarrassment related to the diagnosis, as well as the inconvenience and discomfort of treatment and the fear of recurrence, transmission, and the possible threat of cancer. Co-infections with multiple HPV types are possible and may combine both low-risk and high-risk types, even in cases of genital warts.[7] Great progress has been made recently in control and prevention of HPV infections, and prophylactic HPV vaccines have been used successfully to prevent HPV-related cancers and genital warts.[8] However, genotypes of HPV are over 200 and the distribution of these genotypes differs regionally.[9 10] Although vaccines can protect population in some places, but they can not protect population in other places because of the regional difference of HPV genotype distribution. Thus, information of HPV genotype distribution is of importance for vaccination in a given area.

Actually, HPV infection rates in genital warts varied among reports. Previous multinational study in men from Tampa, Florida, Cuernavaca, Mexico, and Sao Paulo, Brazil reported that the detectable rates of low-risk and high-risk HPV genotypes were 73.2% and 15.6% of external genital lesions in men, respectively.[11] A study in women from Australia found that HPV DNA was detected in 90.8% of genital warts, with HPV 6 and HPV 11 detected in 86.0% of genital warts and high-risk HPV types detected in 31.0% of genital warts.[12] Another study in men in Hong Kong reported that HPV 6/11 was found in 63.1% and HPV 16/18 was found in 9.2% of genital warts.[13] Our study showed that the detectable rates of low-risk and high-risk HPV types were 45.4% and 34.5% in genital warts in Xi'an, China. Although HPV infection rates of genital warts varied among reports, the above-mentioned reports and our study indicated that low-risk HPV types were major causative agents of genital warts, but the prevalence of high-risk HPV types in genital warts was an issue that could not be

ignored. Since genital warts were sexually transmitted diseases, high-risk HPV types in genital warts could act as reservoirs of cancer-related HPV types to threat local population.

The most prevalent genotypes in a given area are the most concerned issue. One study form USA found that HPV 6 (43.8%), HPV 11 (10.7%), and HPV 16 (9.8%) were the genotypes most commonly detected in genital warts in men.[14] Another study from Bogota, Colombia found that HPV 6 was by far the most common type in both women (62%) and men (56%), followed by HPV 11 (20%). HPV16 ranked third in prevalence, with 16% of patients tested positive in genital warts.[15] A study from seven geographical regions of China reported that the most prevalent genotypes were HPV 6 (41.3%), HPV 11 (37.6%) and HPV 16 (10.4%) in genital warts.[16] Our study found that HPV 6 (24.9%), HPV 11 (17.9%), HPV 52 (9.9%) and 16 (7.3%) were the most prevalent HPV types in Xi'an, China. Our data agreed with the report from Guangdong, China, where the most prevalent genotypes were HPV 6 (42.2%), HPV 11 (39.3%), HPV 52 (7.7%), and HPV 16 (7.56%) in patients with genital warts.[17] The above-mentioned reports and our study indicated that HPV 6, 11, and 16 were the most prevalent genotypes in genital warts in most areas of the world. Additionally, HPV 52 was one of the most prevalent genotypes in genital warts in Xi'an and also in Guangdong, China. Whether HPV 52 is one of the most prevalent genotypes in other areas of the world needs to be confirmed.

The gender differences of HPV type prevalence varied among reports. In our study, the detectable rate of low-risk HPV types in men was significantly higher than that in women (52.3% vs 35.7%, P<0.01), while the detectable rate of high-risk HPV in men was not significantly different with that in women (32.4% vs 37.3%, P=0.13). A study from South Africa agreed with our study in that the prevalence of non-carcinogenic HPV in men (33.2%) was higher than that in women (14.0%), but the prevalence of carcinogenic HPV was similar in men (22.4%) and women (22.7%).[18] On the contrary, a study form Liuzhou, China found that the prevalence of nononcogenic HPV types was similar (1.4% vs 1.2%, P=0.6832), whereas the prevalence of oncogenic HPV types was higher in women than in men (18.7% vs 9.4%, P<0.001).[19] At present, it is impossible to summarize the gender differences in HPV type prevalence in genital warts. Widdice et al. suggested that, compared with men, women might have a higher incidence rate or lower clearance rate of HPV infections because of the different physiological structures between genders.[20] Data from Guangdong, China suggested that infected men constituted an important viral reservoir, contributing to transmission of high-risk HPV to women and maintenance of infections, but high-risk HPV infections might be less likely to persist in men than in women.[21] Further prospective studies should be conducted to illustrate the differences in HPV natural history in both sexes.

It also showed in a report that the rate of HPV infections manifested two age-peaks at $\leqslant$25 years and $>$65 years of age. Moreover, most HPV infections among the young patients were transient, and only 5-10% of the infections would become persistent.[22] A recent report from Guiyang, China also observed two age-peaks of HPV infections, one at the age of $\leqslant$20 and the other at the age of 55~59.[23] We found the similar results that low-risk, high-risk and any type HPV detectable rates had 2 age-peaks at the age of 15~19 and 55~59. The age-peaks of HPV infection rate could be explained as patients of these 2 age groups were either too young or too old to have a stronger immunity particularly for protection against HPV infection. Peng et al. suggested that most patients at the first peak got low-risk HPV and caused genital warts, whereas most patients with cervical lesions, including cervical cancer (CC) and cervical intraepithelial neoplasia (CIN), contributed to the second peak at an older age.[23]

Finally, we found that co-infections of HPV types were a common phenomenon in genital warts, as the detectable rates of 1, 2 and $\geqslant$3 HPV types were 26.1%, 17.5% and 14.2%, respectively in our study. The phenomenon of multiple HPV type infections was also found before. For example, Brown et al. used probes for both low- and high-risk HPV types, and they found that 16.3% of genital warts from healthy patients and 52.3% of genital warts form patients with altered cell-mediated immunity, respectively, were positive for both probes.[24] In a retrospective analysis of specimens of women from USA, the results showed that 4.6% of entire sample and 19.0% of HPV-positive sample were positive for 2 or more

HPV types.[25] Data from Italy showed that multiple HPV infections occurred by chance and there was no evidence to show that specific HPV types had the tendency to be found more or less often than others in co-infections.[26] A interesting finding about multiple HPV type infections in genital warts in our study was that the number of infecting HPV types did not accumulate with age increasing, as statistical analysis showed that there was no correlation between infecting HPV type numbers and age.

In conclusion, the present study characterized HPV types in genital warts in Xi'an, China. Our data will provide an important foundation for prevention, diagnosis and treatment of HPV infections and suggest the burden of genital warts can potentially be lowered with increasing usage of vaccination.

Diagnoses of genital warts according to epidemiological history and skin lesions in this study could potentially result in misdiagnosis and missed diagnosis. Patients of genital warts who did not seek treatment and who sought treatment in other clinics in Xi'an were not included. Participants recruited for the study were patients for treatment of genital warts and therefore not a good representative of the general population.

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Contributors The study was designed by CSZ. WHM collected specimens. YFW, HSZ, and JJM performed clinical diagnosis of genital warts. CSZ and YFW analyzed data. CSZ interpreted and drafted the paper. All authors approved the final version of the article.

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STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract (p1)
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found (p1)
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported (p1)
Objectives	3	State specific objectives, including any prespecified hypotheses (p1)
Methods		
Study design	4	Present key elements of study design early in the paper (p2)
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection (p2)
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants(p2)
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable (p2)
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group (p2)
Bias	9	Describe any efforts to address potential sources of bias(p1)
Study size	10	Explain how the study size was arrived at(p2)
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding(p2)
		(*b*) Describe any methods used to examine subgroups and interactions
		(*c*) Explain how missing data were addressed
		(*d*) If applicable, describe analytical methods taking account of sampling strategy
		(*e*) Describe any sensitivity analyses
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed(p3)
		(b) Give reasons for non-participation at each stage(p2)
		(c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders(p3)
		(b) Indicate number of participants with missing data for each variable of interest
Outcome data	15*	Report numbers of outcome events or summary measures(p3-4)
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included(p3-4)
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a

		meaningful time period
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results	18	Summarise key results with reference to study objectives(p5)
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias(p6)
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence(p5-6)
Generalisability	21	Discuss the generalisability (external validity) of the study results(p5-6)
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based(p7)

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Prevalence and distribution of HPV types in genital warts in Xi'an, China: a prospective study

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ABSTRACT

Objectives To characterize the prevalence and distribution of HPV types in genital warts in Xi'an, China.

Methods This prospective study was conducted in Shaanxi Provincial Institute for Skin Disease and STD Control(SPISSC) between September 2014 and April 2017. Genital warts samples were obtained from 879 patients, including 512 men and 367women. HPV genotyping was performed by using an automatic nucleic acid hybridization system.

Results Of the 879 patients of genital warts, the detectable rates of low-risk, high-risk and total HPV types were 45.4%, 34.5% and 57.8%, respectively. The detectable rate of low-risk HPV types (45.4%) was significantly higher than that of high-risk HPV types (34.5%) ($\chi 2=21.85$, $P<0.01$). The detectable rate of low-risk HPV types of men (52.3%) was significantly higher than that of women (35.7%) ($\chi 2=23.90$, $P<0.01$). The detectable rate of one HPV type infections, 2 and 3 or more HPV types co-infections were 26.1%, 17.5% and 14.2%, respectively. HPV6 (24.9%), 11 (17.9%), 52 (9.9%) and 16 (7.3%) were the 4 most common HPV types.

Conclusion Compared with bivalent and quadrivalent vaccines, nonavalent vaccine against HPV6, 11, 16, 18, 31, 33, 45, 52, 58, covering the four most common HPV types(HPV6, 11, 52 and 16), is more suitable for Xi'an, China.

Strengths and limitations of this study

►This is the first study on the prevalence and distribution of HPV types in genital warts in Xi'an, China.

►Diagnosis of genital warts based on clinical manifestations might lead to bias, because infectious soft warts might be misdiagnosed as genital warts due to similar clinical manifestations.

►Some patients with genital warts did not participate because they feared this study might give away their privacy or they visited other convenient hospitals nearby.

INTRODUCTION

Genital warts are a kind of sexually transmitted diseases caused by certain types of human papillomavirus (HPV). The main clinical manifestation of genital warts is benign hyperplasia of the skin and mucous membrane in the genitalia, anus and perineum. Genital warts not only affect physiological function, but also cause psychological stress as well. HPV is the most common sexually transmitted infection globally, and most people are infected at some point in their lives.[1] Genital HPV infections have a prevalence of 10-20% in USA and 1% of the sexually active adults have clinical manifestations.[2] In China, the prevalence of genital HPV infections is 16–18%.[3] To date, 201 different HPV types have been identified. More than 40 of these types can infect mucous membrane of anus and genitalia and have been classified as low-risk (HPV6, 11, 40, 42, 43, 44, 54, 61, 62, 71, 72, 81, 83, 84, 89), probably or possibly high-risk (HPV26, 30, 34, 53, 66, 67, 68, 69, 70, 73, 82, 85, 97) and high-risk (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59) depending on their ability to lead to malignant progression.[4] A variety of HPV types can cause genital warts, but HPV6 and 11 together account for about 90% of all cases.[5] HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82 are considered carcinogenic, but 70% of cervical cancers are caused by HPV16 and 18.[6] Three vaccines have been approved to prevent HPV infections. The vaccines consist of virus-like particles derived from HPV major capsid protein (L1). Cervarix is

bivalent to protect against HPV16 and 18. Gardasil is a recombinant quadrivalent vaccine to protect against HPV6, 11, 16 and 18. Gardasil-9 is nonavalent to protect against HPV6, 11, 16, 18, 31, 33, 45, 52, 58 and can prevent about 90% of cervical, vulvar, vaginal and anal cancers.[7] The reduction of genital warts incidence has been reported in countries where HPV vaccination programs have been adopted.[8] In 2016, China Food and Drug Administration approved HPV vaccine to prevent cervical cancer and other HPV-associated diseases. However, the distribution of HPV types varies from country to country or region to region.[9] At present, the prevalence and distribution of HPV types in genital warts are not clear in Xi'an, China. We conducted this study before implementing an HPV vaccination program. One objective is to characterize the prevalence of HPV infections and distribution of HPV types in Xi'an, China in order to further evaluate the effectiveness of the vaccine, as data showed a decline in vaccine-related HPV types in regions covered by HPV vaccination.[10] Another objective is to assess whether commercially available HPV vaccines can cover the most common HPV types in order to select a suitable vaccine for Xi'an, China.

METHODS

Study design

This study was a prospective study conducted from September 2014 to April 2017. Newly diagnosed patients with genital warts in SPISSC were invited to participate in the study. The specimens were collected from lesions of genital warts and 23 HPV types were detected.

Diagnostic Criteria of Genital Warts

Patients were diagnosed according to the Diagnostic Criteria of Genital Warts of China.[11] Briefly, genital warts were diagnosed based on epidemiological history and clinical manifestations. The epidemiological histories included unsafe sex(es), or sexual partners with genital warts, or multiple sexual partners. The clinical manifestations were papuloid lesions, which might gradually develop into papillary, coronal or cauliflower-like masses. When skin lesions were atypical, acetic acid white test was used to confirm the diagnosis.

Participants

Between September 2014 and April 2017, a total of 912 patients were diagnosed as genital warts in SPISSC. 22 of them were excluded from the study because they were not residents of Xi'an. 11 of them refused to participate because they feared the study might reveal their privacy. The remaining 879 patients, including 512 men and 367 women, participated in this study. Their ages ranged from 16 to 68 and no one had been vaccinated against HPV prior to enrollment.

Specimen collection

We used sampling brushes to collect specimens from lesions of genital warts. The specimens were eluted from the sampling brushes with preservation solution and stored in the refrigerator at -20℃ before detecting HPV types. The sampling brushes and preservation solutions were provided by Yaneng BIOscience (Shenzhen) Co., Ltd.

HPV genotyping

We used an automatic nucleic acid hybridization system manufactured by Yaneng BIOscience (Shenzhen) Co., Ltd to detect HPV types. All procedures were carried out following the manufacturer's instructions. This system used HPV DNA amplification and hybridization to simultaneously detect low-risk HPV6, 11, 42, 43, 81 and high-risk HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 82, 85. Quality control measures were implemented during HPV detection.

Ethical approval

The Ethics Committee of SPISS approved to collect specimens of genital warts and to test the HPV types in the

specimens (approval number: SXEDC2017-001).

Statistical Analysis

All data were analyzed using SPSS software (version 16.0). R×C chi-square test was used for comparing differences among groups, with $\alpha \leq 0.05$ considered statistically significant. A correlation coefficient was calculated to quantify statistical relationship between 2 values, with $\alpha \leq 0.05$ considered significant.

Patient and public involvement

Specimens of patients were collected from lesions of genital warts. Patients were not further involved in the implementation of this study.

RESULTS

Prevalence of low-risk and high-risk HPV types in genital warts

Specimens of 879 patients with genital warts including 512 men and 367 women were tested for low-risk HPV6, 11, 42, 43, 81 and high-risk HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 82, 85. As showed in table 1, of the 512 men, 268 were infected by low-risk (52.3%), 166 by high-risk (32.4%) and 308 by total HPV types (60.2%). Of the 367 women, 131 were infected by low-risk (35.7%), 137 by high-risk (37.3%) and 200 by total HPV types (54.5%). The detectable rate of low-risk HPV types of men (52.3%) was significantly higher than that of women (35.7%) ($\chi 2=23.90$, $P<0.01$), while there was no significant difference in the detection rate of high-risk HPV types between men (32.4%) and women (37.3%) ($\chi 2=2.28$, $P=0.13$). The total detectable rate of low-risk, high-risk, and total HPV types were 45.4%, 34.5% and 57.8%, respectively. The total detectable rate of low-risk HPV types (45.4%) was significantly higher than that of high-risk HPV types (34.5%) ($\chi 2=21.85$, $P<0.01$) .

Table 1. Prevalence of low-risk and high-risk HPV types in genital warts in Xi'an (n=879)

Sex	n	Low-risk(%)	High-risk(%)	Total (%)
Men	512	268 (52.3)	166 (32.4)	308 (60.2)
Women	367	131 (35.7)	137 (37.3)	200 (54.5)
Total	879	399 (45.4)	303 (34.5)	508 (57.8)

Prevalence of low-risk and high-risk HPV types in age groups

As showed in table 2, we divided patients with genital warts into 10 age groups. The detectable rates of low-risk HPV types peaked in the age of 15-19 and 55-59, while that of high-risk HPV types peaked in the age of 55-59.

Table 2. Prevalence of low-risk and high-risk HPVs in age groups in Xi'an (n=879)

Age	n (%)	Low-risk(%)	High-risk(%)	Total (%)
15~	17 (1.9)	10(58.8)	6(35.3)	11(64.7)
20~	152 (17.3)	66 (43.4)	51(33.6)	84(55.3)
25~	232 (26.4)	102(44.0)	81(34.9)	127(54.7)
30~	147 (16.7)	62(42.2)	45(30.6)	77(52.4)
35~	101 (11.5)	46(45.5)	39(38.6)	63(62.4)
40~	88 (10.0)	33 (37.5)	28 (31.8)	48 (54.5)
45~	73 (8.3)	37(50.7)	25(34.2)	48(65.8)

50~	43 (4.9)	25(58.1)	18(41.9)	31(72.1)
55~	15 (1.7)	11(73.3)	7(46.7)	12(80.0)
60~	11 (1.2)	7(63.6)	3(27.3)	7(63.6)
Total	879 (100.0)	399 (45.4)	303 (34.5)	508 (57.8)

Prevalence of each HPV type in genital warts

As showed in table 3, the detectable rate of HPV6 (24.9%), 11 (17.9%), 52 (9.9%), and 16 (7.3%) ranked the top 4 among the 23 HPV types detected. The detectable rate of HPV6 (24.9%) was significantly higher than that of HPV11 (17.9%) ($\chi2$=13.00, P<0.01), while that of HPV11 (17.9%) was significantly higher than that of the HPV52 (9.9%) ($\chi2$=23.32, P<0.01) and that of HPV52 (9.9%) was significantly higher than that of HPV16 (7.3%) ($\chi2$=3.84, P=0.05). So, HPV6, 11, 52 and 16 were the 4 most common HPV types in genital warts in Xi'an, China.

Table 3. Distribution of each HPV types in genital warts in Xi'an (n=879, including 512 men and 367 women)

Type	Men(%)	Women (%)	Total (%)	Rankings
Low-risk				
6	150 (29.3)	69 (18.8)	219(24.9)	1
11	110 (21.5)	47 (12.8)	157(17.9)	2
42	27 (5.3)	20 (5.4)	47(5.3)	5
43	33 (6.4)	14 (3.8)	47(5.3)	5
81	30 (5.9)	16 (4.4)	46(5.2)	6
High-risk				
52	54 (10.5)	33 (9.0)	87(9.9)	3
16	33 (6.4)	31 (8.4)	64(7.3)	4
58	24 (4.7)	22 (6.0)	46(5.2)	6
68	30 (5.9)	9 (2.5)	39(4.4)	7
51	24(4.7)	14(3.8)	38(4.3)	8
56	22(4.3)	16(4.4)	38(4.3)	8
53	18(3.5)	19(5.2)	37(4.2)	9
18	22(4.3)	13(3.5)	35(4.0)	10
66	19(3.7)	12(3.3)	31(3.5)	11
59	17(3.3)	13(3.5)	30(3.4)	12
39	10 (2.0)	9 (2.5)	19(2.2)	13
33	5(1.0)	11(3.0)	16(1.8)	14
31	8(1.6)	6(1.6)	14(1.6)	15
73	9(1.8)	4(1.1)	13(1.5)	16
35	7(1.4)	5(1.4)	12(1.4)	17
85	4(0.9)	3(0.8)	7(0.8)	18
45	5(1.0)	1(0.3)	6(0.7)	19
82	1(0.2)	0 (0.0)	1(0.1)	20
Total	308 (60.2)	200 (54.5)	508(57.8)	--

Multiple HPV type infections in genital warts

As showed in table 4, the detectable rate of 1 HPV type infections was 26.1%, while that of 2 and HPV 3 or more type infections were 17.5% and 14.2%, respectively. The detectable rate of 1 HPV type infections(26.1%) was significantly higher than that of 2 HPV type infections (17.5%) ($\chi 2=18.77$, $P<0.01$) and that of 3 or more HPV type infections (14.2%) ($\chi 2=38.26$, $P<0.01$). Number of HPV types in genital warts varied from 1 to 13. Number of HPV types had a negative correlation with the detectable rate ($r=-0.7258$, $P=0.05$), but there was no correlation between number of HPV types and age ($r=-0.0086$，$P=0.50$).

Table 4. Prevalence of one and multiple HPV type infections in genital warts in Xi'an (n=879)

Number of HPV type	n (%)	Age: $\bar{x} \pm s$
1	229(26.1)	34.9 ± 10.8
2	154(17.5)	32.5 ± 10.5
3	65(7.4)	34.2 ± 10.8
4	20(2.3)	33.5 ± 10.2
5	20(2.3)	30.5 ± 6.8
6	10(1.1)	31.3 ± 14.5
7	8(0.9)	32.0 ± 13.0
8	1(0.1)	34.0 ± 0.0
13	1(0.1)	22.0 ± 0.0
Total	508(57.8)	33.1 ± 10.0

DISCUSSION

Significances to study the prevalence and distribution of HPV types

HPV, as one of the seven notorious carcinogenic viruses, has been studied worldwide. Genital warts are the most common clinical manifestation of HPV infections. Although not life-threatening, genital warts carry a huge psychological and economic burden. Patients with genital warts are disturbed by the shame and embarrassment, and the fear of recurrence and transmission. Co-infections with multiple HPV types are possible and may combine both low-risk and high-risk types.[12] In recent years, significant progress has been made in the control and prevention of HPV infections, and HPV vaccines have been used successfully to prevent HPV-associated cancers and genital warts.[13] However, there are 201 HPV types and their distribution varies from region to region.[14,15] While the vaccines protect people in some areas, they cannot protect people in others because of regional differences in the distribution of HPV types. Therefore, it is important to study the prevalence and distribution of HPV types in specific regions before implementing the vaccination program.

Prevalence of low-risk and high-risk HPV types in genital warts

Prevalence of low-risk and high-risk HPV types in genital warts varied from report to report. A previous multinational study in men reported that the detectable rates of low-risk and high-risk HPV types in genital warts were 73.2% and15.6%, respectively.[16] A study in women from Australia found that HPV was detected in 90.8% of genital warts, with HPV6/11 in 86.0% and high-risk HPV types in 31.0%.[17] Another study in men from Hong Kong reported that HPV6/11 was found in 63.1% and HPV16/18 in 9.2% of genital warts.[18] Our study found that the detectable rates of low-risk and high-risk HPV types were 45.4% and 34.5% in genital warts in Xi'an, China. The above reports and our study indicated that low-risk HPV types are major pathogens of genital warts, but high-risk HPV types in genital warts can act as reservoirs of cancer-related HPV types to threat local population.

The most common HPV types in different regions

One study from USA found that HPV6 (43.8%), 11 (10.7%) and 16 (9.8%) were the most common types detected in genital warts in men.[19] Another study from Colombia found that HPV6 was the most common type in both women (62%) and men (56%), followed by HPV11 (20%). HPV16 ranked third in prevalence, with 16% of patients tested positive in genital warts.[20] A study from seven regions of China reported that the most common types were HPV6 (41.3%), 11 (37.6%) and 16 (10.4%) in genital warts.[21] Our study found that HPV6 (24.9%), 11 (17.9%), 52 (9.9%) and 16 (7.3%) were the most common types in genital warts in Xi'an, China. Consistent with our study, a study from Guangdong, China found that the most common types were HPV6 (42.2%), 11 (39.3%), 52 (7.7%) and 16 (7.56%) in genital warts.[22] The above reports and our study indicate that HPV6, 11, and 16 are the most common types in genital warts in most parts of the world. In addition, HPV52 is one of the most common types in Xi'an and Guangdong, China. Cervarix against HPV16 and 18 does not cover HPV6, 11, 52, while Gardasil against HPV6, 11, 16, 18 does not cover HPV52. Compared with Cervarix and Gardasil, Gardasil-9 against HPV6, 11, 16, 18, 31, 33, 45, 52, 58, covering the four most common HPV types(HPV6, 11, 52 and 16), is more suitable for Xi'an, China.

Sex differences in HPV infection

In our study, the detectable rate of low-risk HPV types of men was significantly higher than that of women (52.3% vs. 35.7%, P<0.01). However, there was no significant difference in the detectable rate of high-risk HPV types between men and women (32.4% vs. 37.3%, P=0.13). Consistent with our study, the prevalence of low-risk HPV types in South African was higher for men (33.2%) than for women (14.0%), but the prevalence of high-risk HPV types was similar for men (22.4%) and women (22.7%).[23] In contrast, a study in Liuzhou, China found no significant difference in the prevalence of low-risk HPV types between men and women (1.2% vs. 1.4%, P=0.68), while the prevalence of high-risk HPV types of men was lower than that of women (9.4% vs.18.7%, P<0.01).[24] Widdice et al suggested that women may have a higher incidence rate or lower clearance rate of HPV infections than men due to their different biological structures between the sexes.[25] The report from Guangdong, China suggested that infected men can transmit HPV to women and high-risk HPV infections may be less likely to persist in men than in women.[26]

Prevalence of low-risk and high-risk HPV types in age groups

Our study found that the detectable rates of low-risk HPV types peaked in the age of 15-19 and 55-59, which can be explained by the fact that patients of these 2 age groups are either too young or too old to have strong immunity to HPV infection. Our study also found that the detectable rates of high-risk HPV types peaked in the age of 55-59. Consistent with our study, Peng et al also observed the rate of HPV infections peaked in the age of ≤20 years (60%) and 55-59 (50.70%). Most patients of the former age group got low-risk HPV types, whereas most patients of the latter age group got high-risk HPV types.[27]

Multiple HPV type infections in genital warts

In this study, we found that multiple HPV type infections in genital warts was common. Brown et al also found multiple HPV type infections in genital warts.[28] A study from USA showed that 19.0% of HPV-positive sample were positive for 2 or more HPV types in women.[29] Data from Italy showed that multiple HPV type infections occurred by chance, and that no particular types of HPV were more likely to occur in co-infections than others.[30] In our study, there was no correlation between number of HPV types and age, suggesting that HPV types do not accumulate with age.

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Contributors CSZ and YFW designed this study and analyzed data. WHM collected specimens and detected HPV types. HSZ and JJM performed clinical diagnosis of genital warts. CSZ drafted the paper. All authors approved the final version of the article.

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STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract (p1)
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found (p1)
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported (p1)
Objectives	3	State specific objectives, including any prespecified hypotheses (p1)
Methods		
Study design	4	Present key elements of study design early in the paper (p2)
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection (p2)
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants(p2)
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable (p2)
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group (p2)
Bias	9	Describe any efforts to address potential sources of bias(p1)
Study size	10	Explain how the study size was arrived at(p2)
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding(p2)
		(*b*) Describe any methods used to examine subgroups and interactions
		(*c*) Explain how missing data were addressed
		(*d*) If applicable, describe analytical methods taking account of sampling strategy
		(*e*) Describe any sensitivity analyses
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed(p3)
		(b) Give reasons for non-participation at each stage(p2)
		(c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders(p3)
		(b) Indicate number of participants with missing data for each variable of interest
Outcome data	15*	Report numbers of outcome events or summary measures(p3-4)
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included(p3-4)
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a

		meaningful time period
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results	18	Summarise key results with reference to study objectives(p5)
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias(p6)
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence(p5-6)
Generalisability	21	Discuss the generalisability (external validity) of the study results(p5-6)
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based(p7)

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Prevalence and distribution of HPV types in genital warts in Xi'an, China: a prospective study

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ABSTRACT

Objectives To characterize the prevalence and distribution of HPV types in genital warts in Xi'an, China.

Methods This prospective study was conducted in Shaanxi Provincial Institute for Skin Disease and STD Control(SPISSC) between September 2014 and April 2017. Genital warts samples were obtained from 879 patients, including 512 men and 367 women. HPV genotyping was performed by using an automatic nucleic acid hybridization system.

Results Of the 879 patients of genital warts, the detectable rates of low-risk, high-risk and total HPV types were 45.4%, 34.5% and 57.8%, respectively. The detectable rate of low-risk HPV types (45.4%) was significantly higher than that of high-risk HPV types (34.5%) ($\chi2=21.85$, $P<0.01$). The detectable rate of low-risk HPV types of men (52.3%) was significantly higher than that of women (35.7%) ($\chi2=23.90$, $P<0.01$). The detectable rate of one HPV type infections, 2 and 3 or more HPV types co-infections were 26.1%, 17.5% and 14.2%, respectively. HPV6 (24.9%), 11 (17.9%), 52 (9.9%) and 16 (7.3%) were the 4 most common HPV types.

Conclusions The results of this study suggest that low-risk HPV types are major pathogens of genital warts, but high-risk HPV type infections and multiple HPV type co-infections are also common in genital warts. HPV6, 11, 52 and 16 are the 4 most common HPV types in genital wart in Xi'an, China.

Strengths and limitations of this study

►This is the first study on the prevalence and distribution of HPV types in genital warts in Xi'an, China.

►Diagnosis of genital warts based on clinical manifestations might lead to bias, because infectious soft warts might be misdiagnosed as genital warts due to similar clinical manifestations.

►Some patients with genital warts did not participate because they feared this study might give away their privacy or they visited other convenient hospitals nearby.

INTRODUCTION

Genital warts are a kind of sexually transmitted diseases caused by certain types of human papillomavirus (HPV). The main clinical manifestation of genital warts is benign hyperplasia of the skin and mucous membrane in the genitalia, anus and perineum. Genital warts not only affect physiological function, but also cause psychological stress as well. HPV is the most common sexually transmitted infection globally, and most people are infected at some point in their lives.[1] Genital HPV infections have a prevalence of 10-20% in USA and 1% of the sexually active adults have clinical manifestations.[2] In China, the prevalence of genital HPV infections is 16–18%.[3] To date, 201 different HPV types have been identified. More than 40 of these types can infect mucous membrane of anus and genitalia and have been classified as low-risk (HPV6, 11, 40, 42, 43, 44, 54, 61, 62, 71, 72, 81, 83, 84, 89), probably or possibly high-risk (HPV26, 30, 34, 53, 66, 67, 68, 69, 70, 73, 82, 85, 97) and high-risk (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59) depending on their ability to lead to malignant progression.[4] A variety of HPV types can cause genital warts, but HPV6 and 11 together account for about 90% of all cases.[5] HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82 are considered carcinogenic, but 70% of cervical cancers are caused by HPV16 and 18.[6] Three vaccines have been approved to prevent

HPV infections. The vaccines consist of virus-like particles derived from HPV major capsid protein (L1). Cervarix is bivalent to protect against HPV16 and 18. Gardasil is a recombinant quadrivalent vaccine to protect against HPV6, 11, 16 and 18. Gardasil-9 is nonavalent to protect against HPV6, 11, 16, 18, 31, 33, 45, 52, 58 and can prevent about 90% of cervical, vulvar, vaginal and anal cancers.[7] The reduction of genital warts incidence has been reported in countries where HPV vaccination programs have been adopted.[8] In 2016, China Food and Drug Administration approved HPV vaccine to prevent cervical cancer and other HPV-associated diseases. However, the distribution of HPV types varies from country to country or region to region.[9] At present, the prevalence and distribution of HPV types in genital warts are not clear in Xi'an, China. We conducted this study before implementing an HPV vaccination program. One objective is to characterize the prevalence of HPV infections and distribution of HPV types in Xi'an, China in order to further evaluate the effectiveness of the vaccine, as data showed a decline in vaccine-related HPV types in regions covered by HPV vaccination.[10] Another objective is to assess whether commercially available HPV vaccines can cover the most common HPV types in order to select a suitable vaccine for Xi'an, China.

METHODS

Study design

This study was a prospective study conducted from September 2014 to April 2017. Newly diagnosed patients with genital warts in SPISSC were invited to participate in the study. The specimens were collected from lesions of genital warts and 23 HPV types were detected.

Diagnostic Criteria of Genital Warts

Patients were diagnosed according to the Diagnostic Criteria of Genital Warts of China.[11] Briefly, genital warts were diagnosed based on epidemiological history and clinical manifestations. The epidemiological histories included unsafe sex(es), or sexual partners with genital warts, or multiple sexual partners. The clinical manifestations were papuloid lesions, which might gradually develop into papillary, coronal or cauliflower-like masses. When skin lesions were atypical, acetic acid white test was used to confirm the diagnosis.

Participants

Between September 2014 and April 2017, a total of 912 patients were diagnosed as genital warts in SPISSC. 22 of them were excluded from the study because they were not residents of Xi'an. 11 of them refused to participate because they feared the study might reveal their privacy. The remaining 879 patients, including 512 men and 367 women, participated in this study. Their ages ranged from 16 to 68 and no one had been vaccinated against HPV prior to enrollment.

Specimen collection

We used sampling brushes to collect specimens from lesions of genital warts. The specimens were eluted from the sampling brushes with preservation solution and stored in the refrigerator at -20℃ before detecting HPV types. The sampling brushes and preservation solutions were provided by Yaneng BIOscience (Shenzhen) Co., Ltd.

HPV genotyping

We used an automatic nucleic acid hybridization system manufactured by Yaneng BIOscience (Shenzhen) Co., Ltd to detect HPV types. All procedures were carried out following the manufacturer's instructions. This system used HPV DNA amplification and hybridization to simultaneously detect low-risk HPV6, 11, 42, 43, 81 and high-risk HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 82, 85. Quality control measures were implemented during HPV detection.

Ethical approval

The Ethics Committee of SPISS approved to collect specimens of genital warts and to test the HPV types in the specimens (approval number: SXEDC2017-001). All participants signed the written informed consent and were informed of their right to withdraw from the study at any time.

Statistical Analysis

All data were analyzed using SPSS software (version 16.0). R×C chi-square test was used for comparing differences among groups, with $\alpha \leq 0.05$ considered statistically significant. A correlation coefficient was calculated to quantify statistical relationship between 2 values, with $\alpha \leq 0.05$ considered significant.

Patient and public involvement

Specimens of patients were collected from lesions of genital warts. Patients were not further involved in the implementation of this study.

RESULTS

Prevalence of low-risk and high-risk HPV types in genital warts

Specimens of 879 patients with genital warts including 512 men and 367 women were tested for low-risk HPV6, 11, 42, 43, 81 and high-risk HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 82, 85. As showed in table 1, of the 512 men, 268 were infected by low-risk (52.3%), 166 by high-risk (32.4%) and 308 by total HPV types (60.2%). Of the 367 women, 131 were infected by low-risk (35.7%), 137 by high-risk (37.3%) and 200 by total HPV types (54.5%). The detectable rate of low-risk HPV types of men (52.3%) was significantly higher than that of women (35.7%) ($\chi2=23.90$, $P<0.01$), while there was no significant difference in the detection rate of high-risk HPV types between men (32.4%) and women (37.3%) ($\chi2=2.28$, $P=0.13$). The total detectable rate of low-risk, high-risk, and total HPV types were 45.4%, 34.5% and 57.8%, respectively. The total detectable rate of low-risk HPV types (45.4%) was significantly higher than that of high-risk HPV types (34.5%) ($\chi2=21.85$, $P<0.01$) .

Table 1. Prevalence of low-risk and high-risk HPV types in genital warts in Xi'an (n=879)

Sex	n	Low-risk(%)	High-risk(%)	Total (%)
Men	512	268 (52.3)	166 (32.4)	308 (60.2)
Women	367	131 (35.7)	137 (37.3)	200 (54.5)
Total	879	399 (45.4)	303 (34.5)	508 (57.8)

Prevalence of low-risk and high-risk HPV types in age groups

As showed in table 2, we divided patients with genital warts into 10 age groups. The detectable rates of low-risk HPV types peaked in the age of 15-19 and 55-59, while that of high-risk HPV types peaked in the age of 55-59.

Table 2. Prevalence of low-risk and high-risk HPVs in age groups in Xi'an (n=879)

Age	n (%)	Low-risk(%)	High-risk(%)	Total (%)
15~	17 (1.9)	10(58.8)	6(35.3)	11(64.7)
20~	152 (17.3)	66 (43.4)	51(33.6)	84(55.3)
25~	232 (26.4)	102(44.0)	81(34.9)	127(54.7)
30~	147 (16.7)	62(42.2)	45(30.6)	77(52.4)
35~	101 (11.5)	46(45.5)	39(38.6)	63(62.4)
40~	88 (10.0)	33 (37.5)	28 (31.8)	48 (54.5)
45~	73 (8.3)	37(50.7)	25(34.2)	48(65.8)
50~	43 (4.9)	25(58.1)	18(41.9)	31(72.1)

55~	15 (1.7)	11(73.3)	7(46.7)	12(80.0)
60~	11 (1.2)	7(63.6)	3(27.3)	7(63.6)
Total	879 (100.0)	399 (45.4)	303 (34.5)	508 (57.8)

Prevalence of each HPV type in genital warts

As showed in table 3, the detectable rate of HPV6 (24.9%), 11 (17.9%), 52 (9.9%), and 16 (7.3%) ranked the top 4 among the 23 HPV types detected. The detectable rate of HPV6 (24.9%) was significantly higher than that of HPV11 (17.9%) ($\chi2$=13.00, P<0.01), while that of HPV11 (17.9%) was significantly higher than that of the HPV52 (9.9%) ($\chi2$=23.32, P<0.01) and that of HPV52 (9.9%) was significantly higher than that of HPV16 (7.3%) ($\chi2$=3.84, P=0.05). So, HPV6, 11, 52 and 16 were the 4 most common HPV types in genital warts in Xi'an, China.

Table 3. Distribution of each HPV types in genital warts in Xi'an (n=879, including 512 men and 367 women)

Type	Men(%)	Women (%)	Total (%)	Rankings
Low-risk				
6	150 (29.3)	69 (18.8)	219(24.9)	1
11	110 (21.5)	47 (12.8)	157(17.9)	2
42	27 (5.3)	20 (5.4)	47(5.3)	5
43	33 (6.4)	14 (3.8)	47(5.3)	5
81	30 (5.9)	16 (4.4)	46(5.2)	6
High-risk				
52	54 (10.5)	33 (9.0)	87(9.9)	3
16	33 (6.4)	31 (8.4)	64(7.3)	4
58	24 (4.7)	22 (6.0)	46(5.2)	6
68	30 (5.9)	9 (2.5)	39(4.4)	7
51	24(4.7)	14(3.8)	38(4.3)	8
56	22(4.3)	16(4.4)	38(4.3)	8
53	18(3.5)	19(5.2)	37(4.2)	9
18	22(4.3)	13(3.5)	35(4.0)	10
66	19(3.7)	12(3.3)	31(3.5)	11
59	17(3.3)	13(3.5)	30(3.4)	12
39	10 (2.0)	9 (2.5)	19(2.2)	13
33	5(1.0)	11(3.0)	16(1.8)	14
31	8(1.6)	6(1.6)	14(1.6)	15
73	9(1.8)	4(1.1)	13(1.5)	16
35	7(1.4)	5(1.4)	12(1.4)	17
85	4(0.9)	3(0.8)	7(0.8)	18
45	5(1.0)	1(0.3)	6(0.7)	19
82	1(0.2)	0 (0.0)	1(0.1)	20
Total	308 (60.2)	200 (54.5)	508(57.8)	--

Multiple HPV type co-infections in genital warts

As showed in table 4, the detectable rate of 1 HPV type infections was 26.1%, while that of 2 and HPV 3 or more type co-infections were 17.5% and 14.2%, respectively. The detectable rate of 1 HPV type infections(26.1%) was significantly higher than that of 2 HPV type co-infections (17.5%) ($\chi2=18.77$, $P<0.01$) and that of 3 or more HPV type co-infections (14.2%) ($\chi2=38.26$, $P<0.01$). Number of HPV types in genital warts varied from 1 to 13. Number of HPV types had a negative correlation with the detectable rate ($r=-0.7258$, $P=0.05$), but there was no correlation between number of HPV types and age ($r=-0.0086$，$P=0.50$).

Table 4. Prevalence of one and multiple HPV type co-infections in genital warts in Xi'an (n=879)

Number of HPV type	n (%)	Age: $\bar{x} \pm s$
1	229(26.1)	34.9 ± 10.8
2	154(17.5)	32.5 ± 10.5
3	65(7.4)	34.2 ± 10.8
4	20(2.3)	33.5 ± 10.2
5	20(2.3)	30.5 ± 6.8
6	10(1.1)	31.3 ± 14.5
7	8(0.9)	32.0 ± 13.0
8	1(0.1)	34.0 ± 0.0
13	1(0.1)	22.0 ± 0.0
Total	508(57.8)	33.1 ± 10.0

DISCUSSION

Significances to study the prevalence and distribution of HPV types

HPV, as one of the seven notorious carcinogenic viruses, has been studied worldwide. Genital warts are the most common clinical manifestation of HPV infections. Although not life-threatening, genital warts carry a huge psychological and economic burden. Patients with genital warts are disturbed by the shame and embarrassment, and the fear of recurrence and transmission. Co-infections with multiple HPV types are possible and may combine both low-risk and high-risk types.[12] In recent years, significant progress has been made in the control and prevention of HPV infections, and HPV vaccines have been used successfully to prevent HPV-associated cancers and genital warts.[13] However, there are 201 HPV types and their distribution varies from region to region.[14,15] While the vaccines protect people in some areas, they cannot protect people in others because of regional differences in the distribution of HPV types. Therefore, it is important to study the prevalence and distribution of HPV types in specific regions before implementing the vaccination program.

Prevalence of low-risk and high-risk HPV types in genital warts

Prevalence of low-risk and high-risk HPV types in genital warts varied from report to report. A previous multinational study in men reported that the detectable rates of low-risk and high-risk HPV types in genital warts were 73.2% and15.6%, respectively.[16] A study in women from Australia found that HPV was detected in 90.8% of genital warts, with HPV6/11 in 86.0% and high-risk HPV types in 31.0%.[17] Another study in men from Hong Kong reported that HPV6/11 was found in 63.1% and HPV16/18 in 9.2% of genital warts.[18] Our study found that the detectable rates of low-risk and high-risk HPV types were 45.4% and 34.5% in genital warts in Xi'an, China. The above reports and our study indicate that low-risk HPV types are major pathogens of genital warts, but high-risk HPV types in genital warts are also common and can act as reservoirs of cancer-related HPV types to threat local population.

The most common HPV types in different regions

One study from USA found that HPV6 (43.8%), 11 (10.7%) and 16 (9.8%) were the most common types detected in genital warts in men.[19] Another study from Colombia found that HPV6 was the most common type in both women (62%) and men (56%), followed by HPV11 (20%). HPV16 ranked third in prevalence, with 16% of patients tested positive in genital warts.[20] A study from seven regions of China reported that the most common types were HPV6 (41.3%), 11 (37.6%) and 16 (10.4%) in genital warts.[21] Our study found that HPV6 (24.9%), 11 (17.9%), 52 (9.9%) and 16 (7.3%) were the most common types in genital warts in Xi'an, China. Consistent with our study, a study from Guangdong, China found that the most common types were HPV6 (42.2%), 11 (39.3%), 52 (7.7%) and 16 (7.56%) in genital warts.[22] The above reports and our study indicate that HPV6, 11, and 16 are the most common types in genital warts in most parts of the world. In addition, HPV52 is one of the most common types in Xi'an and Guangdong, China. Cervarix against HPV16 and 18 does not cover HPV6, 11, 52, while Gardasil against HPV6, 11, 16, 18 does not cover HPV52. Compared with Cervarix and Gardasil, Gardasil-9 against HPV6, 11, 16, 18, 31, 33, 45, 52, 58, covering the 4 most common HPV types(HPV6, 11, 52 and 16), may be more suitable for Xi'an, China.

Sex differences in HPV infection

In our study, the detectable rate of low-risk HPV types of men was significantly higher than that of women (52.3% vs. 35.7%, P<0.01). However, there was no significant difference in the detectable rate of high-risk HPV types between men and women (32.4% vs. 37.3%, P=0.13). Consistent with our study, the prevalence of low-risk HPV types in South African was higher for men (33.2%) than for women (14.0%), but the prevalence of high-risk HPV types was similar for men (22.4%) and women (22.7%).[23] In contrast, a study in Liuzhou, China found no significant difference in the prevalence of low-risk HPV types between men and women (1.2% vs. 1.4%, P=0.68), while the prevalence of high-risk HPV types of men was lower than that of women (9.4% vs.18.7%, P<0.01).[24] Widdice et al suggested that women may have a higher incidence rate or lower clearance rate of HPV infections than men due to their different biological structures between the sexes.[25] The report from Guangdong, China suggested that infected men can transmit HPV to women and high-risk HPV infections may be less likely to persist in men than in women.[26]

Prevalence of low-risk and high-risk HPV types in age groups

Our study found that the detectable rates of low-risk HPV types peaked in the age of 15-19 and 55-59, which can be explained by the fact that patients of these 2 age groups are either too young or too old to have strong immunity to HPV infection. Our study also found that the detectable rates of high-risk HPV types peaked in the age of 55-59. Consistent with our study, Peng et al also observed the rate of HPV infections peaked in the age of ≤20 years (60%) and 55-59 (50.70%). Most patients of the former age group got low-risk HPV types, whereas most patients of the latter age group got high-risk HPV types.[27]

Multiple HPV type co-infections in genital warts

In this study, we found that multiple HPV type co-infections in genital warts was common. Brown et al also found multiple HPV type co-infections in genital warts.[28] A study from USA showed that 19.0% of HPV-positive samples were positive for 2 or more HPV types in women.[29] Data from Italy showed that multiple HPV type co-infections occurred by chance, and that no particular types of HPV were more likely to occur in co-infections than others.[30] In our study, there was no correlation between number of HPV types and age, suggesting that HPV types do not accumulate with age.

CONCLUSIONS

The present study characterized the prevalence and distribution of HPV types in genital warts in Xi'an, China**.** The results suggest that low-risk HPV types are major pathogens of genital warts, but high-risk HPV type infections and multiple HPV type co-infections are also common in genital warts. HPV6, 11, 52 and 16 are the 4 most common HPV

types in genital wart in Xi'an, China and nonavalent vaccine against HPV6, 11, 16, 18, 31, 33, 45, 52, 58, covering the 4 most common HPV types, may be more suitable for Xi'an, China.

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Contributors CSZ and YFW designed this study and analyzed data. WHM collected specimens and detected HPV types. HSZ and JJM performed clinical diagnosis of genital warts. CSZ drafted the paper. All authors approved the final version of the article.

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Competing interests None declared.

Patient consent Obtained.

Provenance and peer review Not commissioned; externally peer reviewed.

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STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract (p1)
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found (p1)
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported (p1)
Objectives	3	State specific objectives, including any prespecified hypotheses (p1)
Methods		
Study design	4	Present key elements of study design early in the paper (p2)
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection (p2)
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants(p2)
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable (p2)
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group (p2)
Bias	9	Describe any efforts to address potential sources of bias(p1)
Study size	10	Explain how the study size was arrived at(p2)
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding(p2)
		(*b*) Describe any methods used to examine subgroups and interactions
		(*c*) Explain how missing data were addressed
		(*d*) If applicable, describe analytical methods taking account of sampling strategy
		(*e*) Describe any sensitivity analyses
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed(p3)
		(b) Give reasons for non-participation at each stage(p2)
		(c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders(p3)
		(b) Indicate number of participants with missing data for each variable of interest
Outcome data	15*	Report numbers of outcome events or summary measures(p3-4)
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included(p3-4)
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a

		meaningful time period
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results	18	Summarise key results with reference to study objectives(p5)
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias(p6)
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence(p5-6)
Generalisability	21	Discuss the generalisability (external validity) of the study results(p5-6)
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based(p7)

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.