4. Menjadi reviewer Jurnal Internasional Bereputasi

KODE K15

DESKRIPSI : Mereview manuskrip di jurnal Scopus Q2, Jurnal Human Vaccines & Immunotherapeutics Halaman

BUKTI :

Permohonan mereview ……. 02

Bukti mereview ke satu ……. 06

Kinerja mereview ke satu …… 07

Respon author dari review ke satu …… 33

Bukti mereview ke dua ……. 93

Kinerja mereview ke dua …… 95

Artikel yang sudah publish …… 111

Permohonan untuk mereview

gondo mastutik <gondomastutik@fk.unair.ac.id>

KHVI-2022-0554 Request to Review from Human Vaccines & Immunotherapeutics

Ronald Ellis <em@editorialmanager.com> Mon, Oct 3, 2022 at 12:39 PM
Reply-To: Ronald Ellis <rellis.hvi@gmail.com>
To: Gondo Mastutik <gondomastutik@fk.unair.ac.id>

Ref.: Ms. No. KHVI-2022-0554
Epidemiology of Human Papillomavirus on Condyloma Acuminatum in Shandong Province，China
Human Vaccines & Immunotherapeutics

Dear Dr. Mastutik:

Human Vaccines & Immunotherapeutics requests your assistance with the peer review process for the recently submitted manuscript entitled ""Epidemiology of Human Papillomavirus on Condyloma Acuminatum in Shandong Province，China " by Haowen Yuan; Renpeng Li; Jian Lv; Guipeng Yi; Xihong Sun; Na zhao; Fengjun Zhao; Aiqiang Xu; Zengqiang Kou; Hongling Wen. We hope that you can complete the review within 18 days of your acceptance to review in order to maintain a timely turn-around time for a decision to the authors. The Editors and Publisher of Human Vaccines & Immunotherapeutics strive to maintain rapid turn-around time while providing useful and constructive comments to authors. The abstract for this manuscript is shown below.

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On behalf of

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Human Vaccines & Immunotherapeutics
Jerusalem 9254113 Israel
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ABSTRACT:

Condyloma acuminatum (CA) is a sexually transmitted disease (STD) caused by human papillomavirus (HPV) infection. It is important to study the prevalence and distribution of HPV genotypes before implementing HPV vaccination program. So, the aim of this study was to evaluate the epidemiological characteristics of CA cases and the distribution of HPV genotypes in Shandong Province, China. One-to-one questionnaire surveys were conducted on all patients diagnosed with CA in sentinel hospitals from Shandong Province, China. HPV genotypes were determined using the polymerase chain reaction (PCR)-reverse dot blot hybridization method. The study enrolled 1185 patients (870 males and 315 females) and found that CA patients are mainly males and sexually active people between the ages of 20 and 40. Recurrence occurred in 411 (34.7%) patients. The overall prevalence of HPV among 880 CA patients undergoing HPV typing was 91.4% (804/880). In these cases, low-risk genotypes predominated, with a prevalence of 91.3% (785/804). High-risk HPV genotypes were found in 430 (53.5%) patients. The most frequent HPV genotypes encountered were HPV 6 (57.8%), HPV 11 (37.2%), HPV 16 (13.7%) and HPV 42 (10.3%). HPV 6 and/or HPV 11 are the main infections in all patients, and more than half of the patients are coinfected with high-risk HPV. However, unlike other regions, HPV42 has a higher prevalence rate among CA patients in Shandong Province and is a nonvaccine HPV genotype. Therefore, regular HPV typing helps to understand the characteristics of specific genotypes and the choice of the best type for vaccine coverage.

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gondo mastutik <gondomastutik@fk.unair.ac.id>

Human Vaccines & Immunotherapeutics - Thank you for agreeing to review KHVI-2022-0554

Human Vaccines & Immunotherapeutics <em@editorialmanager.com> Sat, Oct 8, 2022 at 1:30 PM
Reply-To: Human Vaccines & Immunotherapeutics <khvi-peerreview@journals.tandf.co.uk>
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Human Vaccines & Immunotherapeutics

Review_Due.ics
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Thank you for the review of KHVI-2022-0554

Ronald Ellis <em@editorialmanager.com> Sat, Oct 22, 2022 at 11:03 PM
Reply-To: Ronald Ellis <rellis.hvi@gmail.com>
To: Gondo Mastutik <gondomastutik@fk.unair.ac.id>

Ref.: Ms. No. KHVI-2022-0554
Epidemiology of Human Papillomavirus on Condyloma Acuminatum in Shandong Province，China
Human Vaccines & Immunotherapeutics

Dear Gondo Mastutik,

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Review I

Reviewer Recommendation and Comments for Manuscript Number KHVI-2022-0554

Epidemiology of Human Papillomavirus on Condyloma Acuminatum in Shandong Province, China

Original Submission
Gondo Mastutik Reviewer 2

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Recommendation: Accept with minor revision

Custom Review Question(s):	**Response**
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Would you be willing to review a revision of this manuscript?	No
Do you have any conflict of interest to declare?	No

Reviewer Comments to Author

In general, the manuscript is very well written. The method used to obtain the data is appropriate. Data is displayed correctly. There are only a few corrections in the writing that I wrote in the pdf file.
1. Avoid using abbreviations or numbers at the beginning of sentences.
2. It would be better if you don't mention the number of patients in the table title.
3. How many female who participated in this study? 336 or 315?
4. Please explain why the sample size is different from that in the sub-chapter on sample characteristics and HPV prevalence. 1185 vs 880?

5. Avoid using abbreviations or numbers at the beginning of sentences. It would be better if you change the sentence to something like this: There were 374 (46.5%) patients infected with LR-HPV. Please correct all similar sentences.

6. Please explain why the prevalence of HPV is 91.36%. I think this is not the true prevalence. From the 76 patients whose HPV DNA was negative, was a repeat specimen taken and continued with an HPV genotype examination? I think the negative result in 76 patients is due to insufficient exfoliated cells, so if the number of cells is more or the cell sampling is repeated, the result may be positive. Therefore, in this case, it is better not to mention the prevalence of HPV but the overall HPV DNA is.....

Reviewer Confidential Comments to Editor:

In general, the manuscript is very well written. The method used to obtain the data is appropriate. Data is displayed correctly. Therefore, I suggest accepting this article with a minor correction. So that the author can improve the article properly, please send a pdf file to the author.

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Human Vaccines & Immunotherapeutics

Epidemiology of Human Papillomavirus on Condyloma Acuminatum in Shandong Province , China

--Manuscript Draft--

Manuscript Number:	KHVI-2022-0554
Full Title:	Epidemiology of Human Papillomavirus on Condyloma Acuminatum in Shandong Province , China
Article Type:	Research Article
Order of Authors:	Haowen Yuan
	Renpeng Li
	Jian Lv
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	Aiqiang Xu
	Zengqiang Kou
	Hongling Wen
Keywords:	condyloma acuminatum; human papillomavirus; epidemiology; HPV genotypes
Manuscript Classifications:	115 HPV; 125 Infectious Disease; 80 Epidemiology
Abstract:	Condyloma acuminatum (CA) is a sexually transmitted disease (STD) caused by human papillomavirus (HPV) infection. It is important to study the prevalence and distribution of HPV genotypes before implementing HPV vaccination program. So, the aim of this study was to evaluate the epidemiological characteristics of CA cases and the distribution of HPV genotypes in Shandong Province, China. One-to-one questionnaire surveys were conducted on all patients diagnosed with CA in sentinel hospitals from Shandong Province, China. HPV genotypes were determined using the polymerase chain reaction (PCR)-reverse dot blot hybridization method. The study enrolled 1185 patients (870 males and 315 females) and found that CA patients are mainly males and sexually active people between the ages of 20 and 40. Recurrence occurred in 411 (34.7%) patients. The overall prevalence of HPV among 880 CA patients undergoing HPV typing was 91.4% (804/880). In these cases, low-risk genotypes predominated, with a prevalence of 91.3% (785/804). High-risk HPV genotypes were found in 430 (53.5%) patients. The most frequent HPV genotypes encountered were HPV 6 (57.8%), HPV 11 (37.2%), HPV 16 (13.7%) and HPV 42 (10.3%). HPV 6 and/or HPV 11 are the main infections in all patients, and more than half of the patients are coinfected with high-risk HPV. However, unlike other regions, HPV42 has a higher prevalence rate among CA patients in Shandong Province and is a nonvaccine HPV genotype. Therefore, regular HPV typing helps to understand the characteristics of specific genotypes and the choice of the best type for vaccine coverage.

Epidemiology of Human Papillomavirus on Condyloma Acuminatum in Shandong Province，China

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Abstract

Condyloma acuminatum (CA) is a sexually transmitted disease (STD) caused by human papillomavirus (HPV) infection. It is important to study the prevalence and distribution of HPV genotypes before implementing HPV vaccination program. So, the aim of this study was to evaluate the epidemiological characteristics of CA cases and the distribution of HPV genotypes in Shandong Province, China. One-to-one questionnaire surveys were conducted on all patients diagnosed with CA in sentinel hospitals from Shandong Province, China. HPV genotypes were determined using the polymerase chain reaction (PCR)-reverse dot blot hybridization method. The study enrolled 1185 patients (870 males and 315 females) and found that CA patients are mainly males and sexually active people between the ages of 20 and 40. Recurrence occurred in 411 (34.7%) patients. The overall prevalence of HPV among 880 CA patients undergoing HPV typing was 91.4% (804/880). In these cases, low-risk genotypes predominated,

with a prevalence of 91.3% (785/804). High-risk HPV genotypes were found in 430 (53.5%) patients. The most frequent HPV genotypes encountered were HPV 6 (57.8%), HPV 11 (37.2%), HPV 16 (13.7%) and HPV 42 (10.3%). HPV 6 and/or HPV 11 are the main infections in all patients, and more than half of the patients are coinfected with high-risk HPV. However, unlike other regions, HPV42 has a higher prevalence rate among CA patients in Shandong Province and is a nonvaccine HPV genotype. Therefore, regular HPV typing helps to understand the characteristics of specific genotypes and the choice of the best type for vaccine coverage.

Introduction

Condyloma acuminatum (CA), also known as genital wart (GW), is a verrucous hyperplasia of squamous epithelium in the anogenital region caused by human papillomavirus (HPV) infection, and it is one of the most common sexually transmitted diseases (STDs) [1, 2]. CA is the most common benign lesions of the anogenital region, but it can be difficult to treat and easy to recur, which required repeated treatment [3]. Studies have shown that approximately 48.5% of CA patients will experience recurrence [4]. Because the location of CA is relatively private, the treatment process is painful and expensive, which brings serious psychological pressure and economic burden to the patient, and reduces the quality of life of the patient

[5-7].

At present, more than 200 HPV genotypes have been identified and classified as high-risk (HR) or low-risk (LR), based on oncogenic risk. At least 40 genotypes are associated with infection in the anogenital region [8-10]. HPV 6 and 11 are associated with approximately 90% of CA, whereas 70% of cervical cancers are caused by HPV16 and 18[11]. CA patients are often accompanied by HR-HPV infection [12, 13]. HR-HPV persistent infection is the main pathogenic factor of cervical intraepithelial neoplasia and cervical cancer in women [14, 15].

There is reliable evidence that HPV vaccination has a good preventive effect against HPV infection in uninfected individuals, subsequent CA and HPV-attributable cancers, thereby greatly reducing the burden of HPV-related diseases in patients [16, 17]. However, the HPV vaccines-targeted types are limited, and the HPV genotypes varies among different regions and populations [18].

At present, the HPV infection and epidemiological characteristics of CA patients in China are rare and incomplete. Therefore, in this survey, the evaluation of the epidemic characteristics and HPV genotype distribution of CA cases in Shandong Province, China will contribute to the adjustment of CA prevention and control strategies, the research and development of HPV vaccine and the formulation of immunization plan.

Materials and methods

Study population

Samples were collected from patients diagnosed according to the Diagnosis of Condyloma Acuminatum of China, from Shandong Provincial Hospital for Skin Diseases, Shandong Oriental Andrology Hospital, and Jining No.1 People's Hospital from August 2019 to June 2021. All recruited patients had never been vaccinated against HPV. The selection of sentinel hospitals was based on the number of patients diagnosed with CA in the past.

After accepting a questionnaire administered by trained interviewers, each patient underwent biopsy for HPV typing. Each individual of the patients must volunteer to participate in this study and sign the informed consent. Our study was approved by the ethics committee of school of public health, Shandong University, China (protocol n. 20190320).

Specimen Collection

All samples were collected by uniformly trained clinicians at each clinic visit. Exfoliated cell specimen was collected from the surface of each lesion by using a sampling brush. The brush was rotated 3-5 full turns in clockwise direction to ensure acquisition of the exfoliated cells. For each patient, each specimen was independently eluted from the sampling brush with normal saline and stored in the refrigerator at -80°C before detecting HPV genotypes.

HPV genotyping

HPV tests were performed with the HPV genotyping panel (polymerase chain reaction (PCR)-reverse dot blot hybridization method; Yaneng Biosciences, Shenzhen, China). This multiplex PCR technique can detect 17 high-risk HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73 and 82) and 6 low-risk HPV genotypes (6, 11, 42, 43, 81 and 83). All procedures were carried out following the manufacturer's instructions.

Statistical Analysis

Qualitative categorical variables were described as numbers and percentages. Quantitative variables were described using mean and standard deviation or median and interquartile range (P25- P75), depending on the distribution of the variables. T-test was used to compare quantitative variables with a normal distribution, and Mann-Whitney U-test was used to compare quantitative variables without normal distribution. Chi-square test or Fisher' exact test was conducted to test hypothesis, as appropriate. All hypothesis tests were two-sided, and p-value less than or equal to 0.05 was considered statistically significant. All data were analyzed using SPSS software (version 20.0; IBM Corp.).

Result

CA patients' characteristics

A total of 1185 patients (870 males and 315 females) were included in this study. The ages ranged from 15 to 88, with a mean of 34.85 years (SD = 13.60 years). The mean age varied slightly between males and females (35.90 vs. 31.94 years; P < 0.01). Among recruited patients, 63.7% cases reported more than one sexual partner. The vast majority of patients who reported their sexual preferences were heterosexual (89.1%), including 745 (70.4%) heterosexual men (men who have sex with women, MSW) and 312 (29.5%) heterosexual women. There were 99 men (11.4% of the male patients) who had sex with men (MSM), and 2 women were homosexuals. 26 men and 1 woman who had sex with men and women. Compared with female patients, male patients had more sexual partners (*P* < 0.001). 85.7% of the patients (86.8% of males and 85.0% of females) claimed that their sexual partners were not infected with HPV.

Of the 1185 patients with genital warts, 1 or more subsequent genital warts had been detected in 411 (33.1% of males and 39.0% of females). 32 patients reported other STDs (15 with syphilis, 5 with genital herpes, 6 with HIV/AIDS, 4 with gonorrhea and 2 with chlamydia infection).

The majority of patients stated that only a single body location was infected with warts (53.5%). 34.26% patients presented warts at two different locations, 8.8% at three, and 3.4% at four or more different locations. In females, CA lesions were often located on the urethral orifice / vaginal introitus (62.86% of cases), labia majora / labia minora (35.87%), and

perianal region (26.35%), whereas, for males, the corona sulcus (35.98%), foreskin (26.44%) and perianal region (23.56%) were the most frequent sites.

Table 1. Baseline characteristics of 1185 patients with condyloma acuminatum, by gender

Characteristics	Male (n = 870) n (%)	Female (n= 315) n (%)
Age		
< 18	18 (2.1)	13 (4.1)
18-24	202 (23.2)	99 (31.4)
25-29	120 (13.8)	38 (12.1)
30-39	228 (26.2)	92 (29.2)
40-49	146 (16.8)	38 (12.1)
50-59	93 (10.7)	28 (8.9)
≥60	63 (7.2)	28 (2.2)
Marital status		
Single	319 (36.7)	124 (39.4)
Married	535 (61.1)	182 (57.8)
Widowed/Separated/Divorced	19 (2.2)	9 (2.9)
Education		
Primary/Middle school	225 (25.9)	94 (29.8)
High school	209 (24.0)	49 (15.6)
University/College	419 (48.2)	167 (53.0)
Postgraduate	17 (2.0)	5 (1.6)
Area of residence at diagnosis		
Rural area	263 (30.2)	96 (30.5)
City	607 (69.8)	219 (69.5)
Age of first sexual intercourse		
< 18	76 (8.7)	23 (7.3)
18-21	431 (49.5)	155 (49.2)
≥22	363 (41.7)	137 (43.5)
Type of CA diagnosis		
Recurrent disease	288 (33.1)	123 (39.0)
New diagnosis	582 (75.2)	192 (61.0)
Condom use		
No	108 (12.4)	34 (10.8)
Yes, sometimes/rarely	676 (77.7)	260 (82.5)

Yes, always	86 (9.9)	21 (6.7)
Weekly frequency of sexual		
≤1	394 (45.6)	123 (39.0)
=2	314 (36.1)	118 (37.5)
≥3	159 (18.3)	74 (23.5)
Wash genitalia before sex		
Yes	543 (62.4)	191 (60.6)
No	327 (37.6)	124 (39.4)
Number of sexual partners		
=1	288 (33.1)	142 (45.1)
≥2	582 (66.9)	173 (54.9)

HPV prevalence

In total, 880 samples were collected from 659 males and 221 females with a clinical diagnosis of CA. HPV DNA was detected in 804 of 880 CA cases, yielding an overall prevalence of 91.4% (90.6% males and 93.7% females). 76 samples (from 62 males and 14 females) were excluded from further analysis due to PCR inhibition or insufficient DNA.

Among the 804 patients, 734 (91.3%) patients had at least one LR-HPV infection detected, and 430 (53.5%) patients had HR-HPV infection. 374 (46.5%) patients were only infected with LR-HPV, 70 (8.7%) patients were only infected with HR-HPV, and 360 (44.8%) patients were infected with LR- HPV and HR-HPV. Compared with male patients, female patients had a higher rate of HR-HPV infection ($P < 0.001$). Moreover, co-infection of LR-HPV and HR-HPV was more common in female patients ($P < 0.001$). In addition, the prevalence of single HPV genotype infections was 44.2%, while those of multiple HPV genotype coinfections were 55.8%. The total number of HPV genotypes in patients varied from 1 to 12. The frequency

of single and multiple infections varied significantly by gender, with single infections being more common among males (47.9% males vs. 33.3% females, $P < 0.001$) and multiple infections more common among females (52.1% males vs. 66.7% females, $P < 0.001$). (Table 2)

Table 2. Distribution of infection types in 804 cases of condyloma acuminatum

	Males (n = 597) [a] n (%)	Females (n= 207) [a] n (%)	*P* value
HR-HPV	293(49.1)	137(66.2)	< 0.001
LR-HPV	543(91.0)	191(92.3)	0.563
LR and HR HPV	239 (40.0)	121 (58.5)	< 0.001
Single infection	286 (47.9)	69 (33.3)	< 0.001
Multiple infections	311 (52.1)	138 (66.7)	< 0.001
HPV6 or 11	515 (86.3)	188 (90.8)	0.088
HPV16 or 18	109 (18.3)	49 (23.7)	0.091
Types of 4-valent vaccines [b]	552 (92.5)	196 (94.7)	0.279
Types of 9-valent vaccines [c]	566 (94.8)	200 (96.6)	0.290

[a] Patients with unqualified HPV results were excluded from the analysis.

[b] The types of 4-valent vaccines conclude HPV 6, 11, 16 or 18.

[c] The types of 9-valent vaccines conclude HPV 6, 11, 16, 18, 31, 33, 45, 52 or 58.

Distribution of specific HPV genotypes

Overall, all 23 different HPV genotypes were identified in males and females, with a proportion ranging from 1.5% to 57.8% (12/804 to 465/804). The most frequently encountered HPV genotypes were HPV 6 (57.8%), HPV 11 (37.2%), HPV 16 (13.7%), HPV 42 (10.3%) and HPV 52 (8.8%) by decreasing order of frequency (Figure 1).

As it was shown, HPV 6 (57.8%), HPV 11 (37.2%) and HPV 42 (10.3%) were the three most common LR-HPV genotypes in our study population.

HPV 6 was the most common HPV genotype detected in both males (58.1%) and females (57.0%). HPV 6/11 (alone or in coinfection with other genotypes) were detected in 87.4% patients and there was no significant difference among males and females. In add ition, there was no significant difference in the distribution of all detective low-risk genotypes between males and females. The most common HR-HPV genotypes were HPV 16 (13.7%), HPV 52 (8.8%) and HPV 51 (8.1%). HPV 16/18 (alone or in coinfection with other genotypes) were observed in 19.7% patients. In the HR-HPV genotypes, the frequency of HPV 45, HPV 56 and HPV 58 were significantly higher in women than in men ($P < 0.05$). Among the patients coinfected with LR-HPV and HR-HPV, the most infection mode is HPV6+HPV16 (5.6%). Different frequency of other HPV genotypes in different genders were shown in Table 3.

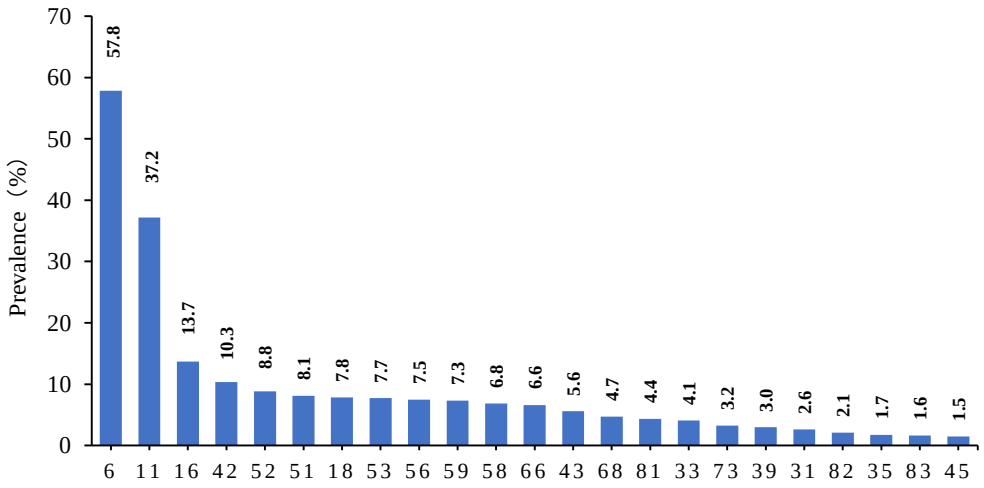

Figure 1 Prevalence of specific human papillomavirus (HPV) infection by decreasing order of frequency. Patients with unqualified HPV results were excluded from the analysis.

Table 3. Prevalence of specific human papillomavirus (HPV) genotypes among 804 cases of

condyloma acuminatum, by decreasing order of frequency.

HPV genotypes	Males (n = 597) [a] n (%)	Females (n= 207) [a] n (%)	*P* value
LR genotypes			
HPV6	347 (58.1)	118 (57.0)	0.779
HPV11	214 (35.8)	85 (41.4)	0.181
HPV42	64 (10.7)	19 (9.2)	0.530
HPV43	37 (6.2)	8 (3.9)	0.208
HPV81	23 (3.9)	12 (5.8)	0.237
HPV83	10 (1.7)	3 (1.4)	0.824
HR genotypes			
HPV16	77 (12.9)	33 (15.9)	0.272
HPV52	51 (8.5)	20 (9.7)	0.625
HPV51	43 (7.2)	22 (10.6)	0.119
HPV18	43 (7.2)	20 (9.7)	0.257
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HPV31	16 (2.7)	5 (2.4)	0.837
HPV82	12 (2.0)	5 (2.4)	0.727
HPV35	8 (1.3)	6 (2.9)	0.140
HPV45	5 (0.8)	7 (3.4)	0.009

[a] Patients with unqualified HPV results were excluded from the analysis.

Discussion

As we all know, CA is one of the most common sexually transmitted infections (STIs) caused by HPV infection, and most sexually active persons are usually exposed to HPV during their lifetime [19]. In this study based on surveys in sentinel hospitals, we reported the prevalent characteristics of CA and distribution of HPV genotypes in patients with CA in Shandong Province, China. In our study, a total of 1185 CA patients

were recruited. The recruited patients were mainly males. This may be due to the differences in the anatomical structure of males and females. Compared with males, females need to undergo a professional gynecological examination in the hospital to find out whether they have CA. The average age of CA patients recruited in this study was 34.85 years. Compared with men, women were younger. These results are consistent with a previous Chinese multi-center study of genital wart conducted in 2013 including 1,005 cases from eighteen hospitals in seven geographical regions of China [20]. In this study, most patients were in the sexually active stage aged 20 to 40. These patients had risky sexual behaviors such as multiple sexual partners and irregular condom use. Among them, 32 cases also suffered from other sexually transmitted diseases. These are all risk factors that increase HPV infection [21, 22]. In addition, 34.7% of the patients in this study were recurrences.

Among the 880 patients who underwent HPV testing, the overall HPV prevalence rate was 91.36%, which shows that HPV is the pathogen that causes CA. 8.6% of the patients could not be included in the analysis due to insufficient cellular DNA content. Some studies have found that 16~26% of the samples will be negative regardless of the type of cell brush sampling [23, 24]. Compared with other studies, the lower negative rate of our study reflects the higher quality of sample collection after unified training. In the analysis of specific HPV types, it was shown that the most common

genotypes in Shandong Province, China were HPV6, HPV11, HPV16 and HPV42. Compared with other regions in China, HPV42 had a higher prevalence in Shandong province and had become one of the common genotypes of HPV in CA patients. Previous studies have found that the distribution of HPV genotypes varies from region to region. Although the risk of diseases related to nonvaccine HPV genotypes is theoretically still low, the actual situation still needs to be monitored to deal with the disease burden caused by high prevalence of nonvaccine HPV genotypes. Furthermore, the distribution of HPV6, HPV11 and HPV16 were consistent with the results of studies in other regions of China [20, 25, 26]. In this study, males were mainly infected with low-risk HPV, while females were mainly multiple infected with high-risk and low-risk HPV. Most patients, both male and female, were infected with more than one different HPV genotype. According to our results, HPV6 and 11 (alone or in coinfection with other genotypes) were detected in 87.4% of patients, but most of these patients also had high-risk HPV infection. In this study, 53.5% of patients with CA were infected with high-risk HPV. 49.1% of male patients were infected with at least one high-risk type of HPV. Some studies have shown that male penile cancer and anal cancer are associated with high-risk HPV infection [27]. Compared with male patients, female patients had a higher infection rate of high-risk HPV (66.2% vs. 49.1%), indicating that women had a higher burden of high-risk HPV infection.

Persistent high-risk HPV infection can lead to precancerous lesions and cancers of the cervix, vulva, vagina, and anus in women, so it is very important to prevent the occurrence of these lesions [28]. Some studies find that high HPV vaccination coverage significantly reduced the prevalence of CA, HPV infection and cervical intraepithelial neoplasia grade 2+ (CIN2+) [29, 30]. In addition, when formulating policies to prevent HPV infection in women, it should be considered that men also have a higher infection rate of high-risk HPV. Some studies have found that men HPV multiple infection is very common, and the high-risk HPV infection rate is the same as that of women [31-33]. There is a higher risk of cross-infection between men and women [34, 35]. HPV vaccination to men can effectively reduce related HPV infection rates and HPV-related cancer incidence [36, 37].

Nowadays, four prophylactic HPV vaccines are available in China, including the HPV bivalent vaccine (Cervarix®; Cecolin®) to protect against HPV16 and 18; the HPV quadrivalent vaccine (Gardasil®) to protect against HPV 6, 11, 16 and 18; the HPV nonavalent vaccine (Gardasil®9) to protect against HPV 6, 11, 16, 18, 31, 33, 45, 52 and 58 [38, 39]. At present, there is no HPV vaccination programs implemented in China, which may be related to the insufficient data of HPV epidemiological survey. However, the distribution of HPV genotypes varies from region to region [40]. Although vaccines can provide

protection for people, it is important to study the prevalence and distribution of HPV genotypes in different regions before implementing the HPV vaccination plan, because understanding the distribution of HPV genotypes will help to choose the best vaccine for HPV protection in each region. In this study, HPV6 and HPV11 were the causes of CA in the majority of patients (87.4%), and they were included in both the quadrivalent and nonavalent vaccines. Considering the economic benefits and the insufficient supply of 9-valent HPV vaccine in China, the 4-valent HPV vaccine may be more suitable for the prevention of CA in Shandong Province, China. Furthermore ,HPV42, as one of the genotypes with higher prevalence in this study, has not been included in any licensed HPV vaccines. Previous studies have found that among people vaccinated with the quadrivalent HPV vaccine, the infection rate of nonvaccine HPV genotypes is remaining flat or higher [41]. Therefore, it is necessary to develop a broader spectrum of HPV vaccines to reduce the disease burden caused by HPV infection [42].

In conclusion, CA cases are mainly infected with low-risk HPV, but there are more high-risk HPV infections in both males and females, which is a problem that cannot be ignored in clinical treatment. It is necessary to monitor the HPV genotype of CA patients, because high-risk HPV infection is the main risk factor for cancer. For the general public, HPV typing and HPV vaccination will help to prevent CA or HPV-related cancer

and reduce the risk of disease. Additionally, nonvaccine HPV genotypes also need to be monitored for their prevalence by regular HPV typing. The results of our study provide a contribution to the epidemiology of HPV in Chinese CA patients with improving the understanding of existing HPV genotypes. Furthermore, these will have a positive impact on the adjustment of CA prevention and treatment strategies, the research and development of HPV vaccines and the formulation of immunization programs.

Acknowledgments

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Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Funding

This work has been funded by a research grant from Merck Sharpe & Dohme (MISP IIS#57766).

Ethics and dissemination

Our study was approved by the ethics committee of School of Public Health, Shandong University, China (protocol n. 20190320). Individuals must voluntarily agree to participate and must sign the free and informed consent form.

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figure 1

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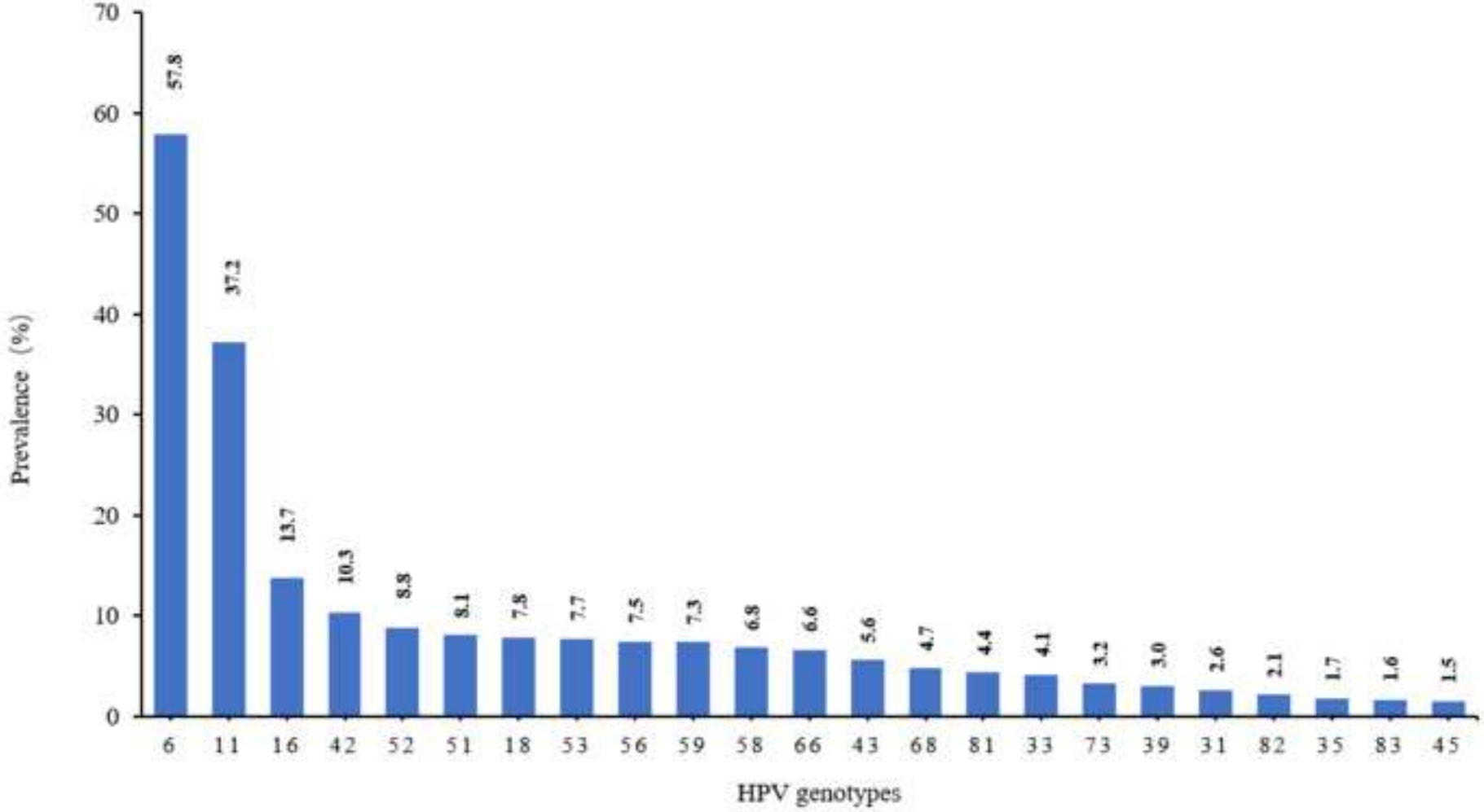

Table 1. Baseline characteristics of 1185 patients with condyloma acuminatum, by gender

Characteristics	Male (n = 870) n (%)	Female (n= 315) n (%)
Age		
< 18	18 (2.1)	13 (4.1)
18-24	202 (23.2)	99 (31.4)
25-29	120 (13.8)	38 (12.1)
30-39	228 (26.2)	92 (29.2)
40-49	146 (16.8)	38 (12.1)
50-59	93 (10.7)	28 (8.9)
≥60	63 (7.2)	28 (2.2)
Marital status		
Single	319 (36.7)	124 (39.4)
Married	535 (61.1)	182 (57.8)
Widowed/Separated/Divorced	19 (2.2)	9 (2.9)
Education		
Primary/Middle school	225 (25.9)	94 (29.8)
High school	209 (24.0)	49 (15.6)
University/College	419 (48.2)	167 (53.0)
Postgraduate	17 (2.0)	5 (1.6)
Area of residence at diagnosis		
Rural area	263 (30.2)	96 (30.5)
City	607 (69.8)	219 (69.5)
Age of first sexual intercourse		
< 18	76 (8.7)	23 (7.3)
18-21	431 (49.5)	155 (49.2)
≥22	363 (41.7)	137 (43.5)
Type of CA diagnosis		
Recurrent disease	288 (33.1)	123 (39.0)
New diagnosis	582 (75.2)	192 (61.0)
Condom use		
No	108 (12.4)	34 (10.8)
Yes, sometimes/rarely	676 (77.7)	260 (82.5)
Yes, always	86 (9.9)	21 (6.7)
Weekly frequency of sexual		
≤1	394 (45.6)	123 (39.0)
=2	314 (36.1)	118 (37.5)
≥3	159 (18.3)	74 (23.5)
Wash genitalia before sex		

Yes	543 (62.4)	191 (60.6)
No	327 (37.6)	124 (39.4)
Number of sexual partners		
=1	288 (33.1)	142 (45.1)
≥2	582 (66.9)	173 (54.9)

Table 2. Distribution of infection types in 804 cases of condyloma acuminatum

	Males (n = 597) [a] n (%)	Females (n= 207) [a] n (%)	*P* value
HR-HPV	293(49.1)	137(66.2)	< 0.001
LR-HPV	543(91.0)	191(92.3)	0.563
LR and HR HPV	239 (40.0)	121 (58.5)	< 0.001
Single infection	286 (47.9)	69 (33.3)	< 0.001
Multiple infections	311 (52.1)	138 (66.7)	< 0.001
HPV6 or 11	515 (86.3)	188 (90.8)	0.088
HPV16 or 18	109 (18.3)	49 (23.7)	0.091
Types of 4-valent vaccines [b]	552 (92.5)	196 (94.7)	0.279
Types of 9-valent vaccines [c]	566 (94.8)	200 (96.6)	0.290

[a] Patients with unqualified HPV results were excluded from the analysis.

[b] The types of 4-valent vaccines conclude HPV 6, 11, 16 or 18.

[c] The types of 9-valent vaccines conclude HPV 6, 11, 16, 18, 31, 33, 45, 52 or 58.

Table 3. Prevalence of specific human papillomavirus (HPV) genotypes among 804 cases of condyloma acuminatum, by decreasing order of frequency.

HPV genotypes	Males (n = 597) [a] n (%)	Females (n= 207) [a] n (%)	*P* value
LR genotypes			
HPV6	347 (58.1)	118 (57.0)	0.779
HPV11	214 (35.8)	85 (41.4)	0.181
HPV42	64 (10.7)	19 (9.2)	0.530
HPV43	37 (6.2)	8 (3.9)	0.208
HPV81	23 (3.9)	12 (5.8)	0.237
HPV83	10 (1.7)	3 (1.4)	0.824
HR genotypes			

HPV16	77 (12.9)	33 (15.9)	0.272
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[a] Patients with unqualified HPV results were excluded from the analysis.

Jawaban Author dari comment review I

Human Vaccines & Immunotherapeutics

Epidemiology of Human Papillomavirus on Condyloma Acuminatum in Shandong Province , China

--Manuscript Draft--

Manuscript Number:	KHVI-2022-0554R1
Full Title:	Epidemiology of Human Papillomavirus on Condyloma Acuminatum in Shandong Province , China
Article Type:	Research Article
Order of Authors:	Haowen Yuan
	Renpeng Li
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	Aiqiang Xu
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Keywords:	condyloma acuminatum; human papillomavirus; epidemiology; HPV genotypes
Manuscript Classifications:	115 HPV; 125 Infectious Disease; 80 Epidemiology
Abstract:	Condyloma acuminatum (CA) is a sexually transmitted disease (STD) caused by human papillomavirus (HPV) infection. It is important to study the prevalence and distribution of HPV genotypes before implementing HPV vaccination program. Therefore, the aim of this study was to evaluate the epidemiological characteristics of CA cases and the distribution of HPV genotypes in Shandong Province, China. One-to-one questionnaire surveys were conducted on all patients diagnosed with CA in sentinel hospitals from Shandong Province, China. HPV genotypes were determined using the polymerase chain reaction (PCR)-reverse dot blot hybridization method. The study enrolled 1185 patients (870 males and 315 females) and found that CA patients are mainly males and sexually active people between the ages of 20 and 40. Recurrence occurred in 34.7% patients. Among the 880 CA patients who underwent HPV typing, the HPV test positivity rate was 91.4%. In these cases, low-risk HPV infection was predominant, with an infection rate of 91.3%, while high-risk HPV genotypes were found in 53.5% patients. The most frequent HPV genotypes encountered were HPV 6 (57.8%), HPV 11 (37.2%), HPV 16 (13.7%) and HPV 42 (10.3%). HPV 6 and/or HPV 11 are the main infections in all patients, and more than half of the patients are coinfected with high-risk HPV. However, unlike other regions, HPV42 has a higher prevalence rate among CA patients in Shandong Province and is a nonvaccine HPV genotype. Therefore, regular HPV typing helps to understand the characteristics of specific genotypes and the choice of the best type for vaccine coverage.
Response to Reviewers:	

Ronald Ellis PhD MBA
Editor-in-Chief
Human Vaccines & Immunotherapeutics

2022.12.16

Dear Prof Ellis,

Re: Manuscript reference No. KHVI-2022-0554
220855034

Please find attached a revised version of our manuscript "**Epidemiology of Human Papillomavirus on Condyloma Acuminatum in Shandong Province, China**", which we would like to resubmit for publication as a research article in Human Vaccines & Immunotherapeutics.

Your comments and those of the reviewers were highly insightful and enabled us to greatly improve the quality of our manuscript. In the following pages are our point-by-point responses to each of the comments of the reviewers as well as your own comments. The reviewer's comments and questions are shown in bold.

We have edited and revised the manuscript using the "Track Changes" tool in Microsoft Word. We have also quoted the articles recommended by you and reviewers. We hope that the revisions in the manuscript and our accompanying responses will be sufficient to make our manuscript suitable for publication in Human Vaccines & Immunotherapeutics.

We shall look forward to hearing from you at your earliest convenience.

Yours sincerely,
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Reviewers' comments and questions:

Editorial Comments

1. Remove the Tables from the manuscript DOC and upload as a separate DOC file.

Response: Thanks for your careful reading and checks. We have removed the Tables from the manuscript DOC and uploaded it as a separate Tables DOC file.

2. Remove the Figures from the manuscript DOC file.

Response: Thanks for your careful reading. We have removed the Figures from the manuscript DOC and uploaded it as a separate Figures DOC file.

3. Avoid using abbreviations or numbers at the beginning of sentences.

Response: Thank you very much for your careful reading. We have fixed these errors.

4. Do not mention the number of patients in the Table title.

Response: We thanks the reviewer for pointing this out. We have removed the number of patients from the Table title. Please review the separate Tables DOC file.

5. Round all numbers to the nearest 0.1 not 0.01.

Response: We appreciate your careful reading. We have corrected these errors.

Major Comments

1. Materials and Methods

a. The part of HPV DNA testing is written too simply. Add specific PCR amplification steps.

Response: Thanks a lot for the reviewer kind suggestion. Under your guidance, we added the specific PCR amplification steps in the part of HPV DNA testing. Please see page 6-7 of the revised manuscript, lines 127–143.

All HPV DNA extraction, amplification and genotype identification were performed with the HPV genotyping panel (polymerase chain reaction (PCR)-reverse dot blot hybridization method; Yaneng Biosciences, Shenzhen, China). The HPV genotyping panel can detect 17 high-risk HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73 and 82) and 6 low-risk HPV genotypes (6, 11, 42, 43, 81 and 83). Genomic DNA was extracted from specimens following manufacturer's protocols. The PCR cycling conditions were as follows: initial denaturation at 95°C for 5 min, then 40 cycles at 94°C for 30 s, 42°C for 90 s, 72°C for 30 s, and a final extension at 72°C for 5 min. The HPV type-specific probes are immobilized on nylon membranes for reverse-blot hybridization, which can detect 23 types of HPV genotypes in a single test. Hybridization was performed and visualized by assessing the protocol according to the manufacturer's instructions. The quality and integrity of the extracted DNA is

monitored by the β-actin gene assay. The specimens with known HPV genotypes were used as positive controls and enzyme-free water was used as a negative control.

b. Describe in detail the examination and specific sampling sites of men and women, such as whether the men or women who received anal sex had anal canal examined and specimen collection. Did women receive the vagina and cervix exam? Was the oral cavity examined for people who had active oral sex?
Conduct statistical analysis on the difference of HPV infection in different specimen collection sites.
Response: Thanks for your professional comments. Anal and oral specimens were not collected from all patients with acromegaly in this study, only the site where the CA lesions were located in the patient was examined and samples were collected. Some of these patients had CA lesions located in the anal area. We describe the process of collecting specimens in more detail in Sample Collection. Please see page 6 of the revised manuscript, lines 115–119. We also found that few studies have reported in detail on anal and oral HPV infection in patients with condyloma acuminatum. Your valuable comments will provide us with valuable ideas to improve our next studies.

c. Explain why 1185 patients were enrolled at the beginning of the study, but only 880 patients were tested for HPV typing, why were the remaining 305 patients not tested for HPV typing？

Response: Thank you very much for your careful reading and your professional comments. A total of 1185 patients in this study consented to the questionnaire. Of these, 880 consented to the sample collection while the remaining 305 patients did not consent to the sample collection and they only consented to the questionnaire.

d. There are three times as many male as female subjects. Does this have any impact on the distribution difference of HPV types between men and women in subsequent analysis?
Response: We appreciate your careful reading and critical comments. The aim of this study was to explore the prevalence characteristics and HPV genotypes distribution of CA cases in Shandong Province, China. We believe that the sample size included in this study is large enough to reflect the current true prevalence of condyloma acuminatum in Shandong Province, China. In addition, previous studies have found that the sex ratio of male to female patients with condyloma acuminatum in China has been increasing year by year, with more male patients than female patients. The patient profile included in this study is consistent with the epidemiological characteristics of patients with condyloma acuminatum in China, and is in line with the findings of relevant studies in China and abroad. Therefore, we do not believe that there will be an impact on the differences in the distribution of HPV types between males and females in the subsequent analysis.
References:
1. Yue X，Gong X，Li J，et al. Epidemiological features of condyloma acuminatum

in national sexually transmitted disease surveillance sites in China from 2008 to 2016 [J]. Chinese Journal of Dermatology,2017,50(05):321-325.
2. Zhu C, Wang Y, Mao W, et al. Prevalence and distribution of HPV types in genital warts in Xi'an, China: a prospective study[J]. BMJ Open,2019,9(5):e23897.
3. Azevedo J, Pista A, Lisboa C, et al. Epidemiology of human papillomavirus on anogenital warts in Portugal - The HERCOLES study[J]. Journal of the European Academy of Dermatology and Venereology,2017,31(8):1342-1348.

2. Results

a. Explain why the prevalence of HPV is 91.4%, which appears incorrect. From the 76 patients whose HPV DNA was negative, was a repeat specimen taken and continued with an HPV genotype examination? The negative result in 76 patients may be due to insufficient exfoliated cells, so if the number of cells is more or the cell sampling is repeated, the result may be positive. Thus in this case, it is better not to mention the prevalence of HPV but the overall HPV DNA is ....

Response: Thank you very much for your careful reading and valuable comments. Samples that tested negative in this study were retested for confirmation. Insufficient cellular DNA was not included in the analysis mainly due to the cases were β-globin negative. In a review of genital HPV infection in men, the authors reported that, regardless of the type of cytobrush used, the proportion of β-globin-negative samples ranged from 16% to 26%, and our results were lower than in their study (8.6%).

We have changed "HPV DNA was detected in 804 of 880 CA cases, yielding an overall prevalence of 91.4% (90.6% males and 93.7% females)" to "The HPV DNA was detected in 804 of the 880 CA cases, with a positive rate of 91.4% (90.6% males and 93.7% females)". Please see page 10 of the revised manuscript, lines 204–206.

References:

1. Partridge J M, Koutsky L A. Genital human papillomavirus infection in men[J]. The Lancet Infectious Diseases,2006,6(1):21-31.

b. Table1, marital status, education, urban or rural areas, and the time of the first sexual act, etc., should be further analyzed.

Response: Thanks a lot for the reviewer kind suggestion. The aim of this study was to evaluate the prevalence characteristics and HPV genotype distribution of CA cases in Shandong Province, China. CA cases from sentinel hospitals between August 2019 and June 2021 were collected to illustrate the epidemiological characteristics of condyloma acuminatum in Shandong Province, China. Thank you very much for your suggestion and we will take these factors you suggested into account in a follow-up study to further explore the impact of factors such as marital status, education, urban or rural area, and the time of first sexual intercourse on the prevalence characteristics of CA.

c. Table 2 has a similar number of people between 4v and 9v vaccines. Can it be shown that the protection rate of the quadrivalent vaccine is similar to that of the nine-valent vaccine? In the absence of an expensive nine-valent vaccine, the cost performance of quadrivalent vaccine is higher for individual and group

prevention and control.

Response: We appreciate the reviewer's insightful question. All of the subjects in our study were patients with condyloma acuminatum and the predominant types of infection were HPV6 and HPV11. Because both the quadrivalent and nine-valent vaccines cover HPV6 and HPV11, the number of people infected with the HPV genotypes covered by the quadrivalent and nine-valent vaccines is similar. Both quadrivalent and nine-valent vaccines reduce the prevalence of CA, but it cannot be inferred from the similar number of infections that they have similar protection rates; this requires further clinical trials to obtain the protection rates for both vaccines.

d. In "HPV prevalence", line 7 mentions that 374 cases were only infected with LR-HPV and 70 cases were only infected with HR-HPV, which was slightly reflected in Table 2. Clarify this discrepancy.

Response: We appreciate your careful reading and critical comments. Only infected with LR-HPV means that only low-risk HPV tests are positive and all high-risk HPV tests are negative. Similarly, only infected with HR-HPV means that only the high-risk HPV types tested positive and all the low-risk HPV types tested negative. We have added this section to Table 2 in the separate Tables DOC file.

e. 34.7% of the patients had relapse. Is relapse related to HPV typing? Correlation analysis should be done.

Response: Thanks for your professional comments. There were 215 relapsed patients and 589 non-relapsed patients who received HPV testing. A correlation analysis was performed between recurrence and HPV type, but the results found no correlation between recurrence and HPV type, so the results were not added to the manuscript. The results are as follows:

Schedule 1 Correlation of CA recurrence with HPV genotypes

HPV genotypes	Recurrence (n = 215) *N* (%)	No recurrence (n= 589) *N* (%)	*P* value
LR genotypes			
HPV6	125 (58.1)	340 (57.7)	0.916
HPV11	76 (35.3)	223 (37.9)	0.514
HPV42	21 (9.8)	62 (10.5)	0.754
HPV43	10 (4.7)	35 (5.9)	0.481
HPV81	5 (2.3)	30 (5.1)	0.089
HPV83	6 (2.8)	7 (1.2)	0.111
HR genotypes			
HPV16	29 (13.5)	81 (13.8)	0.923
HPV52	21 (9.8)	50 (8.5)	0.572
HPV51	13 (6.0)	52 (8.8)	0.200
HPV18	12 (5.6)	51 (8.7)	0.151
HPV53	15 (7.0)	47 (8.0)	0.637
HPV56	14 (6.5)	46 (7.8)	0.535
HPV59	17 (7.9)	42 (7.1)	0.709

HPV58	18 (8.4)	37 (6.3)	0.299
HPV66	20 (9.3)	33 (5.6)	0.061
HPV68	8 (3.7)	30 (5.1)	0.417
HPV33	7 (3.3)	26 (4.4)	0.464
HPV73	6 (2.8)	20 (3.4)	0.668
HPV39	8 (3.7)	16 (2.7)	0.459
HPV31	7 (3.3)	14 (2.4)	0.489
HPV82	5 (2.3)	12 (2.0)	0.801
HPV35	2 (0.9)	12 (2.0)	0.288
HPV45	3 (1.4)	9 (1.5)	0.891

f. State how informed consent was obtained for minors under the age of 18?
Response: Thanks for your nice question. We first obtain the consent of the guardian of a minor under the age of 18 and then the consent of the patient, and only then do we carry out the investigation. Please see page 5-6 of the revised manuscript, lines 109–111.

g. Confirm that the subjects underwent biopsy for HPV typing.
Response: Thanks for the reviewer good suggestion. We think although the cytobrush was probably not the best tool for cell collection, it was surely the most appropriate, because it was noninvasive and represented an easy and acceptable sampling method for patients.

h. "85.7% of the patients (86.8% of males and 85.0% of females) claimed that their sexual partners were not infected with HPV". This part of the data is inconsistent with the clinical data. Are the data obtained by the patient's oral description or confirmed by laboratory testing?
Response: We appreciate your careful reading and critical comments. This part of the data was obtained through face-to-face interviews with the patients. As the patient had more than one number of sexual partners, there were some difficulties in performing laboratory tests on the patient's sexual partners, which made it difficult to obtain consent from the patient and his/her sexual partners. However, this study did require knowledge of the HPV status of the patient's sexual partners, so it was only possible to find out from the patient the infection status of his or her regular sexual partners. Please see page 9 of the revised manuscript, lines 180–182.

i. "32 patients reported other STDs". This part of the data is inconsistent with the clinical data. How did you get these data？

Response: Thanks for your question. In this study, each patient was examined not only for condyloma acuminatum, but also for other sexually transmitted diseases. This data is obtained by further laboratory tests on the patient.

j. Explain what "a single body location" means.

Response: Thanks for your careful reading. "a single body location" means that only one body site has the condyloma acuminatum lesions. We have modified “The majority of patients stated that only a single body location was infected with warts (53.5%). 34.26% patients presented warts at two different locations, 8.8% at three, and 3.4% at four or more different locations” to “The majority of patients presented warts lesions at one body site (53.5%), 34.3% patients presented warts at two different sites, 8.8% at three, and 3.4% at four or more different sites”. Please see page 9 of the revised manuscript, lines 188–192.

3. Discussion

a. The article mentions that compared with other areas of China, Shandong Province HPV42 in patients with condyloma acuminatum prevalence is higher. Do you have data on the prevalence of HPV42 in other regions?

Also, explain whether additional HPV42 vaccine is necessary in the context of virulence and other relevant data.

Response: Thanks for your nice comments. The prevalence of HPV42 in other regions of China was obtained from previously published studies. Currently, the prevalence of HPV42 is not high throughout mainland China, and is only relatively high in Shandong Province. It may not be economically appropriate to develop an additional HPV42 vaccine. If an additional HPV42 vaccine is needed, this may require more epidemiological data as a supporting basis, as well as the need for regular surveillance of the prevalence of non-vaccine covered HPV genotypes.

References:

1. Zhu C, Wang Y, Mao W, et al. Prevalence and distribution of HPV types in genital warts in Xi’an, China: a prospective study[J]. BMJ Open,2019,9(5):e23897.

2. Luo Z, Chen Q, Yang H, et al. The Prevalence and Genotype of Human Papillomavirus from Patients with Genital Warts in Eastern Guangdong Province[J]. Asian Pacific Journal of Cancer Prevention,2015,16(14):5675-5679.

3. Wang H, Qiao Y L. Human papillomavirus type-distribution in condylomata acuminata of mainland China: a meta-analysis[J]. International Journal of STD & AIDS,2008,19(10):680-684.

b. "8.6% of the patients could not be included in the analysis due to insufficient cellular DNA content": Have you considered other reasons?

Response: Thanks for your good question. "8.6% of patients could not be included in the analysis due to insufficient cellular DNA content" was mainly due to a negative result for β-globin during the test. In a review of genital HPV infection in men, the authors reported that, regardless of the type of cytobrush used, the proportion of β-globin-negative samples ranged from 16% to 26%, and our results were lower than in their study (8.6%).

References:

1. Partridge J M, Koutsky L A. Genital human papillomavirus infection in men[J]. The Lancet Infectious Diseases,2006,6(1):21-31.

Minor Comments

1. Clarify how many females participated in this study? 336 or 315?
Response: We apologize for our error and thanks for your careful reading. There were 315 women who participated in this study. We have corrected the error in Table 1 in the separate Tables DOC file.

2. Line 34 paragraph 1. Change "So" to "Therefore".
Response: Thanks for your good suggestion. We have changed "So" to "Therefore". Please see page 2 of the revised manuscript, lines 34.

3. Line 57, paragraph 1. Change "genital wart" to "genital warts".
Response: We thanks the reviewer for pointing this out. We have corrected the typo. Please see page 3 of the revised manuscript, lines 63.

4. Lines 64-6. Correct the grammar in "Because the location of CA is relatively private, the treatment economic burden to the patient, and reduces the quality of life of the patient". A sentence should not be composed of two clauses, in other words, a sentence should have a body sentence. Furthermore, better to write the sentence more concrete. For instance, you could write "the treatment process is painful and the treatment fee is expensive."
Response: We appreciate your careful reading and critical comments. We have changed "Because the location of CA is relatively private, the treatment process is painful and expensive, which brings serious psychological pressure and economic burden to the patient, and reduces the quality of life of the patient" to "The occurrence of CA reduces the quality of life of patients. On the one hand, the affected area of CA is private and the treatment process is painful, which brings serious psychological pressure to patients. On the other hand, the treatment fee is expensive, which brings heavy financial burden to patients and their families.". Please see page 4 of the revised manuscript, lines 70–74.

5. Lines 91-2, diagnosed according to ... - Attach the year of the guideline, and cite the guideline in reference.
Response: Thanks for your careful reading. We have attached the year of the guideline, and cited the guideline in reference. Please see page 5 of the revised manuscript, lines 103–104.
References:
1. Chinese Society of Dermatology and Venereology，China Dermatologist Association, Chinese Association of Rehabilitation Dermatology and Venereology. Guideline for the Clinical Management of Anogenital Warts in China (2014) [J]. Chinese Journal of Dermatology, 2014,47(08):598-599.

6. Line 100, Revise "must volunteer to". "Must" means that you have to do something while volunteer means you want to do something without other people's

intention or supervision.
Response: We apologize for our error and thanks the reviewer for pointing this out. We have changed "Each individual of the patients must volunteer to participate in this study and sign the informed consent." to "All patients volunteered to participate in this study and signed the informed consent (Minors under the age of 18 have the consent of their guardians)". Please see page 6 of the revised manuscript, lines 113–115.

7. Line 98. Filling in a questionnaire is more idiomatic than "accepting a questionnaire".
Response: According to your valuable comments, we have changed "accepting a questionnaire" to "filling in a questionnaire". Please see page 5 of the revised manuscript, lines 110.

8. Line 103. Report how many samples were collected from each hospital.
Response: Thanks for your professional comments. Shandong Dermatology Hospital collected 407 samples, while Jining No.1 People's Hospital and Shandong Oriental Anorectal Hospital collected 321 and 152 samples, respectively. The number of samples collected from each hospital has been described in the Results section. Please see page 11 of the revised manuscript, lines 206–208.

9. Line 137, Among recruited patients, 63.7% cases reported having more than one sexual partner.
Add "having" to make the sentence complete.
Response: Thanks for your careful reading and checks. We have revised our manuscript according to your suggestions. Please see page 8 of the revised manuscript, lines 175.

10. Line 142. From US CDC 2021 STIs Guideline, the term "MSM" often is used clinically to refer to sexual behavior alone, regardless of sexual orientation (e.g., a person might identify as heterosexual but still be classified as MSM). In your study, you reported that 26 men and 1 woman had sex with men and women.
Classify the 26 men as MSM for their sexual behavior.
Response: Thanks for your professional comments. This study needed to know the detailed sexual behavior of each patient, not just the sexual orientation. Therefore, the bisexual group was further delineated. The 26 males and 1 female should be classified as bisexual because they had sex with both males and females. We have changed "There were 99 men (11.4% of the male patients) who had sex with men (MSM), and 2 women were homosexuals. 26 men and 1 woman who had sex with men and women" to "There were 101 patients who were homosexual, including 99 males (men who had sex with men, MSM) and 2 females. In addition, 26 males and 1 female were bisexual who had sex with both males and females". Please see page 9 of the revised manuscript, lines 178-181.

11. Lines 151-2. In "CA patients' characteristics", the retention of the decimal point is inconsistent.

Response: We appreciate your careful reading. We have corrected these errors. Please see page 9-10 of the revised manuscript, lines 193-200.

12. Line 216, CA is one of the most common STDs. For the term "sexually transmitted infection" (STI) refers to a pathogen that causes infection through sexual contact, whereas the term "sexually transmitted disease" (STD) refers to a recognizable disease state that has developed from an infection.

Response: According to your valuable comments, we have changed "sexually transmitted infection" to "sexually transmitted disease". Please see page 14 of the revised manuscript, lines 267.

13. Line 276, Is it feasible for men to be vaccinated with HPV vaccine in case of vaccine shortage?

Response: We think that HPV vaccination for men is feasible in the face of vaccine shortages. Previous studies have found that HPV vaccination in men significantly reduces the prevalence of anal cancer, condyloma acuminata, and other diseases. In addition, there is a good immunization effect with only one dose.

References:

1. Chow E P F, Tabrizi S N, Fairley C K, et al. Prevalence of human papillomavirus in young men who have sex with men after the implementation of gender-neutral HPV vaccination: a repeated cross-sectional study[J]. The Lancet Infectious Diseases,2021.

2. Goldstone S E, Giuliano A R, Palefsky J M, et al. Efficacy, immunogenicity, and safety of a quadrivalent HPV vaccine in men: results of an open-label, long-term extension of a randomised, placebo-controlled, phase 3 trial[J]. The Lancet Infectious Diseases,2021.

3. Basu P, Malvi S G, Joshi S, et al. Vaccine efficacy against persistent human papillomavirus (HPV) 16/18 infection at 10 years after one, two, and three doses of quadrivalent HPV vaccine in girls in India: a multicentre, prospective, cohort study[J]. The Lancet Oncology,2021,22(11):1518-1529.

14. Line 312. "make" a contribution. "make" is more idiomatic than "provide".

Response: We thanks the reviewer for pointing this out. We have corrected these errors. Please see page 19 of the revised manuscript, lines 363.

15. Table 1

a. variable "weekly frequency of sexually" seems like an uncompleted expression.

b. male vertical column, 394+314+159=867.Please explain why it is not 870.

c. variable "type of CA diagnosis", male vertical column, 33.1%+75.2%=108.3%. Explain why it is not 100%.

Response: We apologize for our errors and thanks for your careful reading. We have corrected these errors in Table 1 in the separate Tables DOC file.

Epidemiology of Human Papillomavirus on Condyloma Acuminatum in Shandong Province，China

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Abstract

Condyloma acuminatum (CA) is a sexually transmitted disease (STD) caused by human papillomavirus (HPV) infection. It is important to study the prevalence and distribution of HPV genotypes before implementing HPV vaccination program. Therefore, the aim of this study was to evaluate the epidemiological characteristics of CA cases and the distribution of HPV genotypes in Shandong Province, China. One-to-one questionnaire surveys were conducted on all patients diagnosed with CA in sentinel hospitals from Shandong Province, China. HPV genotypes were determined using the polymerase chain reaction (PCR)-reverse dot blot hybridization method. The study enrolled 1185 patients (870 males and 315 females) and found that CA patients are mainly males and sexually active people between the ages of 20 and 40. Recurrence occurred in 34.7% patients. Among the 880 CA patients who underwent HPV typing, the HPV test positivity rate was 91.4%. In these cases, low-risk HPV genotypes infection was predominant,

with an infection rate of 91.3%, while high-risk HPV genotypes were found in 53.5% patients. The most frequent HPV genotypes encountered were HPV 6 (57.8%), HPV 11 (37.2%), HPV 16 (13.7%) and HPV 42 (10.3%). HPV 6 and/or HPV 11 are the main infections in all patients, and more than half of the patients are coinfected with high-risk HPV. However, unlike other regions, HPV42 has a higher prevalence rate among CA patients in Shandong Province and is a nonvaccine HPV genotype. Therefore, regular HPV typing helps to understand the characteristics of specific genotypes and the choice of the best type for vaccine coverage.

Keywords: condyloma acuminatum; human papillomavirus; epidemiology; HPV genotypes

Introduction

Condyloma acuminatum (CA), also known as genital warts (GW), is a verrucous hyperplasia of squamous epithelium in the anogenital region caused by human papillomavirus (HPV) infection, and it is one of the most common sexually transmitted diseases (STDs) [1, 2]. The CA is the most common benign lesions of the anogenital region, but it can be difficult to treat and easy to recur, which required repeated treatment [3]. Studies have shown that approximately 48.5% of CA patients will experience recurrence [4]. The occurrence of CA reduces the quality of life of patients. On the one hand, the affected area of CA is private and the treatment process is

painful, which brings serious psychological pressure to patients. On the other hand, the treatment fee is expensive, which brings heavy financial burden to patients and their families. [5-7].

At present, more than 200 HPV genotypes have been identified and classified as high-risk (HR) or low-risk (LR), based on oncogenic risk. At least 40 genotypes are associated with infection in the anogenital region [8-10]. HPV 6 and 11 are associated with approximately 90% of CA, whereas 70% of cervical cancers are caused by HPV16 and 18[11]. Patients with CA are often accompanied by HR-HPV infection [12-15]. Persistent HR-HPV infection is the main pathogenic factor of cervical intraepithelial neoplasia and cervical cancer in women [16,17].

There is reliable evidence that HPV vaccination has a good preventive effect against HPV infection in uninfected individuals, subsequent CA and HPV-attributable cancers, thereby greatly reducing the burden of HPV-related diseases in patients [18, 19]. However, the HPV vaccines-targeted types are limited, and the HPV genotypes varies among different regions and populations [20].

At present, the HPV infection and epidemiological characteristics of CA patients in China are rare and incomplete. Therefore, in this survey, the evaluation of the epidemic characteristics and HPV genotype distribution of CA cases in Shandong Province, China will contribute to the adjustment of CA prevention and control strategies, the research and development of

HPV vaccine and the formulation of immunization plan.

Materials and methods

Study population

Participants were recruited from patients diagnosed according to the Guideline for the Clinical Management of Anogenital Warts in China (2014) [21], from Shandong Provincial Hospital for Skin Diseases, Shandong Oriental Andrology Hospital, and Jining No.1 People's Hospital from August 2019 to June 2021. All recruited patients had never been vaccinated against HPV. The selection of sentinel hospitals was based on the number of patients diagnosed with CA in the past.

After filling in a questionnaire administered by trained interviewers, each patient underwent sample collection for HPV typing. All patients volunteered to participate in this study and signed the informed consent (Minors under the age of 18 had the consent of their guardians). Our study was approved by the ethics committee of school of public health, Shandong University, China (protocol n. 20190320).

Specimen Collection

All patients undergo a clinical examination by a trained clinician at each visit to determine whether there are CA lesions in the cervical, vaginal, vulva, anal and perianal areas in women or in the external genital, anal and perianal sites in men. If the CA lesion is found, the sample will be collected

from the site by clinician. Exfoliated cell specimen was collected from the surface of each CA lesion by using a sampling brush. The brush was rotated 3-5 full turns in clockwise direction to ensure acquisition of the exfoliated cells. For each patient, each specimen was independently eluted from the sampling brush with normal saline and stored in the refrigerator at -80°C before detecting HPV genotypes.

HPV genotyping

All HPV DNA extraction, amplification and genotype identification were performed with the HPV genotyping panel (polymerase chain reaction (PCR)-reverse dot blot hybridization method; Yaneng Biosciences, Shenzhen, China). The HPV genotyping panel can detect 17 high-risk HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73 and 82) and 6 low-risk HPV genotypes (6, 11, 42, 43, 81 and 83). Genomic DNA was extracted from specimens following manufacturer's protocols. The PCR cycling conditions were as follows: initial denaturation at 95°C for 5 min, then 40 cycles at 94°C for 30 s, 42°C for 90 s, 72°C for 30 s, and a final extension at 72°C for 5 min. The HPV type-specific probes are immobilized on nylon membranes for reverse-blot hybridization on, which can detect 23 types of HPV genotypes in a single test. Hybridization was performed and visualized by assessing the protocol according to the manufacturer's instructions. The quality and integrity of the extracted DNA is monitored by the β-actin gene assay. The specimens with known HPV

genotypes were used as positive controls and enzyme-free water was used as a negative control.

Statistical Analysis

Qualitative categorical variables were described as numbers and percentages. Quantitative variables were described using mean and standard deviation or median and interquartile range (P25- P75), depending on the distribution of the variables. *T*-test was used to compare quantitative variables with a normal distribution, and Mann-Whitney *U*-test was used to compare quantitative variables without normal distribution. Chi-square test or Fisher' exact test was conducted to test hypothesis, as appropriate. All hypothesis tests were two-sided, and *P*-value less than or equal to 0.05 was considered statistically significant. All data were analyzed using SPSS software (version 20.0; IBM Corp.).

Result

CA patients' characteristics

A total of 1185 patients (870 males and 315 females) were included in this study. Shandong Provincial Hospital for Skin Diseases contributed 52.8% of the total participants, whilst 34.4% and 12.8% were from Jining No.1 People's Hospital and Shandong Oriental Andrology Hospital, respectively. The ages ranged from 15 to 88, with a mean of 34.85 years (SD = 13.60 years). The mean age varied slightly between males and females (35.90 vs.

31.94 years; $P < 0.01$). Among recruited patients, 63.7% cases reported having more than one sexual partner. The vast majority of patients who reported their sexual preferences were heterosexual (89.1%), including 745 (70.4%) heterosexual men (men who have sex with women, MSW) and 312 (29.5%) heterosexual women. There were 101 patients who were homosexual, including 99 males (men who had sex with men, MSM) and 2 females. In addition, 26 males and 1 female were bisexual who had sex with both males and females. Compared with female patients, male patients had more sexual partners ($P < 0.001$). In this study, 85.7% of the patients (86.8% of males and 85.0% of females) themselves claimed that their sexual partners were not infected with HPV.

Of the 1185 patients with genital warts, one or more subsequent genital warts had been detected in 411 patients (33.1% of males and 39.0% of females). In addition, there have 32 patients reported other STDs (Fifteen with syphilis, five with genital herpes, six with HIV/AIDS, four with gonorrhea and two with chlamydia infection).

The majority of patients presented warts lesions at one location (53.5%), 34.3% patients presented warts at two different locations, 8.8% at three, and 3.4% at four or more different locations. In females, the CA lesions were often located on the urethral orifice / vaginal introitus (62.9% of cases), labia majora / labia minora (35.9%), and perianal region (26.4%), whereas, for males, the corona sulcus (36.0%), foreskin (26.4%) and

perianal region (23.6%) were the most frequent sites.

HPV prevalence

A total of 880 samples were collected from 659 males and 221 females with a clinical diagnosis of CA. Shandong Dermatology Hospital collected 407 samples, while Jining No.1 People's Hospital and Shandong Oriental Anorectal Hospital collected 321 and 152 samples, respectively. The HPV DNA was detected in 804 of the 880 CA cases, with a positive rate of 91.4% (90.6% males and 93.7% females). Seventy-six samples (from 62 males and 14 females) were excluded from further analysis due to PCR inhibition or insufficient DNA.

Among the 804 patients, 734 (91.3%) patients had at least one LR-HPV infection detected, and 430 (53.5%) patients had HR-HPV infection. In addition, there were 374 (46.5%) patients only infected with LR-HPV, 70 (8.7%) patients were only infected with HR-HPV, and 360 (44.8%) patients were infected with both LR- HPV and HR-HPV. Compared with male patients, female patients had a higher rate of HR-HPV infection ($P < 0.001$). Moreover, co-infection of LR-HPV and HR-HPV was more common in female patients ($P < 0.001$). In addition, the prevalence of single HPV genotype infections was 44.2%, while those of multiple HPV genotype coinfections were 55.8%. The total number of HPV genotypes in patients varied from 1 to 12. The frequency of single and multiple infections varied

significantly by gender, with single infections being more common among males (47.9% males vs. 33.3% females, $P < 0.001$) and multiple infections more common among females (52.1% males vs. 66.7% females, $P < 0.001$). (Table 2)

Distribution of specific HPV genotypes

Overall, all 23 different HPV genotypes were identified in males and females, with a proportion ranging from 1.5% to 57.8% (12/804 to 465/804). The most frequently encountered HPV genotypes were HPV 6 (57.8%), HPV 11 (37.2%), HPV 16 (13.7%), HPV 42 (10.3%) and HPV 52 (8.8%) by decreasing order of frequency. (Figure 1)

As it was shown, HPV 6 was the most common HPV genotype detected in both males (58.1%) and females (57.0%). HPV 6/11 (alone or in coinfection with other genotypes) were detected in 87.4% patients and there was no significant difference among males and females. In addition, there was no significant difference in the distribution of all detective low-risk genotypes between males and females. The most common HR-HPV genotypes were HPV 16 (13.7%), HPV 52 (8.8%) and HPV 51 (8.1%). HPV 16/18 (alone or in coinfection with other genotypes) were observed in 19.7% patients. In the HR-HPV genotypes, the frequency of HPV 45, HPV 56 and HPV 58 were significantly higher in women than in men ($P < 0.05$). Among the patients coinfected with LR-HPV and HR-HPV, the

most infection mode is HPV6+HPV16 (5.6%). Different frequency of other HPV genotypes in different genders were shown in Table 3.

Discussion

As we all know, CA is one of the most common sexually transmitted diseases (STDs) caused by HPV infection, and most sexually active persons are usually exposed to HPV during their lifetime [22]. In this study based on surveys in sentinel hospitals, we reported the prevalent characteristics of CA and distribution of HPV genotypes in patients with CA in Shandong Province, China. In our study, a total of 1185 CA patients were recruited. The recruited patients were mainly males. This may be due to the differences in the anatomical structure of males and females. Compared with males, females need to undergo a professional gynecological examination in the hospital to find out whether they have CA. The average age of CA patients recruited in this study was 34.85 years. Compared with men, women were younger. These results are consistent with a previous Chinese multi-center study of genital wart conducted in 2013 including 1,005 cases from eighteen hospitals in seven geographical regions of China [23]. In this study, most patients were in the sexually active stage aged 20 to 40. These patients had risky sexual behaviors such as multiple sexual partners and irregular condom use. Among them, 32 cases also suffered from other sexually transmitted diseases. These are all

risk factors that increase HPV infection [24, 25]. In addition, 34.7% of the patients in this study were recurrences.

Among the 880 patients who underwent HPV testing, the overall HPV test positivity rate was 91.4%, which shows that HPV is the pathogen that causes CA. There were 8.6% of the patients could not be included in the analysis due to insufficient cellular DNA content. Previous studies have found that 16~26% of the samples will be negative regardless of the type of cell brush sampling [26, 27]. Compared with other studies, the lower negative rate of our study reflects the higher quality of sample collection after unified training. In the analysis of specific HPV types, it was shown that the most common genotypes in Shandong Province, China were HPV6, HPV11, HPV16 and HPV42. Compared with other regions in China, HPV42 had a higher prevalence in Shandong province and had become one of the common genotypes of HPV in CA patients. Previous studies have found that the distribution of HPV genotypes varies from region to region. Although the risk of diseases related to nonvaccine HPV genotypes is theoretically still low, the actual situation still needs to be monitored to deal with the disease burden caused by high prevalence of nonvaccine HPV genotypes. Furthermore, the distribution of HPV6, HPV11 and HPV16 were consistent with the results of studies in other regions of China [23, 28, 29].

In this study, males were mainly infected with low-risk HPV, while females

were mainly multiple infected with high-risk and low-risk HPV. Most patients, both male and female, were infected with more than one different HPV genotype. According to our results, HPV6 and 11 (alone or coinfection with other genotypes) were detected in 87.4% of patients, but most of these patients also had high-risk HPV infection. In this study, 53.5% of patients with CA were infected with high-risk HPV. Among male patients, 49.1% were infected with at least one high-risk type of HPV. Some studies have shown that male penile cancer and anal cancer are associated with high-risk HPV infection [30]. Compared with male patients, female patients had a higher infection rate of high-risk HPV (66.2% vs. 49.1%), indicating that women had a higher burden of high-risk HPV infection. Persistent high-risk HPV infection can lead to precancerous lesions and cancers of the cervix, vulva, vagina, and anus in women, so it is very important to prevent the occurrence of these lesions [31]. Some studies find that high HPV vaccination coverage significantly reduced the prevalence of CA, HPV infection and cervical intraepithelial neoplasia grade 2+ (CIN2+) [32, 33]. In addition, when formulating policies to prevent HPV infection in women, it should be considered that men also have a higher infection rate of high-risk HPV. Some studies have found that men HPV multiple infection is very common, and the high-risk HPV infection rate is the same as that of women [34-36]. There is a higher risk of cross-infection between men and women [37, 38]. HPV vaccination to

men can effectively reduce related HPV infection rates and HPV-related cancer incidence [39, 40].

Nowadays, four prophylactic HPV vaccines are available in China, including the HPV bivalent vaccine (Cervarix®; Cecolin®) to protect against HPV16 and 18; the HPV quadrivalent vaccine (Gardasil®) to protect against HPV 6, 11, 16 and 18; the HPV nonavalent vaccine (Gardasil®9) to protect against HPV 6, 11, 16, 18, 31, 33, 45, 52 and 58 [41, 42]. At present, there is no HPV vaccination programs implemented in China, which may be related to the insufficient data of HPV epidemiological survey. However, the distribution of HPV genotypes varies from region to region [43]. Although vaccines can provide protection for people, it is important to study the prevalence and distribution of HPV genotypes in different regions before implementing the HPV vaccination plan, because understanding the distribution of HPV genotypes will help to choose the best vaccine for HPV protection in each region [44]. In this study, HPV6 and HPV11 were the causes of CA in the majority of patients (87.4%), and they were included in both the quadrivalent and nonavalent vaccines. Considering the economic benefits and the insufficient supply of 9-valent HPV vaccine in China, the 4-valent HPV vaccine may be more suitable for the prevention of CA in Shandong Province, China. Furthermore ,HPV42, as one of the genotypes with higher prevalence in this study, has not been included in any licensed HPV

vaccines. Previous studies have found that among people vaccinated with the quadrivalent HPV vaccine, the infection rate of nonvaccine HPV genotypes is remaining flat or higher [45]. Therefore, it is necessary to develop a broader spectrum of HPV vaccines to reduce the disease burden caused by HPV infection [46].

In conclusion, CA cases are mainly infected with low-risk HPV, but there are more high-risk HPV infections in both males and females, which is a problem that cannot be ignored in clinical treatment. It is necessary to monitor the HPV genotype of CA patients, because high-risk HPV infection is the main risk factor for cancer. For the general public, HPV typing and HPV vaccination will help to prevent CA or HPV-related cancer and reduce the risk of disease. Additionally, nonvaccine HPV genotypes also need to be monitored for their prevalence by regular HPV typing. The results of our study make a contribution to the epidemiology of HPV in Chinese CA patients with improving the understanding of existing HPV genotypes. Furthermore, these will have a positive impact on the adjustment of CA prevention and treatment strategies, the research and development of HPV vaccines and the formulation of immunization programs.

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Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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Ethics and dissemination

Our study was approved by the ethics committee of School of Public Health, Shandong University, China (protocol n. 20190320). All patients volunteered to participate in this study and signed the informed consent (Minors under the age of 18 had the consent of their guardians).

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Epidemiology of Human Papillomavirus on Condyloma Acuminatum in Shandong Province，China

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Abstract

Condyloma acuminatum (CA) is a sexually transmitted disease (STD) caused by human papillomavirus (HPV) infection. It is important to study the prevalence and distribution of HPV genotypes before implementing HPV vaccination program. ~~So~~Therefore, the aim of this study was to evaluate the epidemiological characteristics of CA cases and the distribution of HPV genotypes in Shandong Province, China. One-to-one questionnaire surveys were conducted on all patients diagnosed with CA in sentinel hospitals from Shandong Province, China. HPV genotypes were determined using the polymerase chain reaction (PCR)-reverse dot blot hybridization method. The study enrolled 1185 patients (870 males and 315 females) and found that CA patients are mainly males and sexually active people between the ages of 20 and 40. Recurrence occurred in ~~411 (~~34.7%~~)~~ patients. Among the 880 CA patients who underwent HPV typing, the HPV test positivity rate was 91.4%.~~The overall prevalence of HPV among 880~~

~~CA patients undergoing HPV typing was 91.4% (804/880).~~ In these cases, low-risk HPV infection was predominant, with an infection rate of 91.3%, while high-risk HPV genotypes were found in 53.5% patients.~~In these cases, low-risk genotypes predominated, with a prevalence of 91.3% (785/804). High-risk HPV genotypes were found in 430 (53.5%) patients.~~ The most frequent HPV genotypes encountered were HPV 6 (57.8%), HPV 11 (37.2%), HPV 16 (13.7%) and HPV 42 (10.3%). HPV 6 and/or HPV 11 are the main infections in all patients, and more than half of the patients are coinfected with high-risk HPV. However, unlike other regions, HPV42 has a higher prevalence rate among CA patients in Shandong Province and is a nonvaccine HPV genotype. Therefore, regular HPV typing helps to understand the characteristics of specific genotypes and the choice of the best type for vaccine coverage.

Keywords: condyloma acuminatum; human papillomavirus; epidemiology; HPV genotypes

Introduction

Condyloma acuminatum (CA), also known as ~~genital wart~~genital warts (GW), is a verrucous hyperplasia of squamous epithelium in the anogenital region caused by human papillomavirus (HPV) infection, and it is one of the most common sexually transmitted diseases (STDs) [1, 2]. The CA is the most common benign lesions of the anogenital region, but it can be

difficult to treat and easy to recur, which required repeated treatment [3]. Studies have shown that approximately 48.5% of CA patients will experience recurrence [4]. The occurrence of CA reduces the quality of life of patients. On the one hand, the affected area of CA is private and the treatment process is painful, which brings serious psychological pressure to patients. On the other hand, the treatment fee is expensive, which brings heavy financial burden to patients and their families.~~Because the location of CA is relatively private, the treatment process is painful and expensive, which brings serious psychological pressure and economic burden to the patient, and reduces the quality of life of the patient~~ [5-7].

At present, more than 200 HPV genotypes have been identified and classified as high-risk (HR) or low-risk (LR), based on oncogenic risk. At least 40 genotypes are associated with infection in the anogenital region [8-10]. HPV 6 and 11 are associated with approximately 90% of CA, whereas 70% of cervical cancers are caused by HPV16 and 18[11]. Patients with CA~~CA patients~~ are often accompanied by HR-HPV infection [12-15~~, 13~~]. Persistent HR-HPV ~~persistent~~ infection is the main pathogenic factor of cervical intraepithelial neoplasia and cervical cancer in women [~~14~~16, ~~15~~17].

There is reliable evidence that HPV vaccination has a good preventive effect against HPV infection in uninfected individuals, subsequent CA and HPV-attributable cancers, thereby greatly reducing the burden of HPV-

related diseases in patients [~~16~~18, ~~17~~19]. However, the HPV vaccines-targeted types are limited, and the HPV genotypes varies among different regions and populations [~~18~~20].

At present, the HPV infection and epidemiological characteristics of CA patients in China are rare and incomplete. Therefore, in this survey, the evaluation of the epidemic characteristics and HPV genotype distribution of CA cases in Shandong Province, China will contribute to the adjustment of CA prevention and control strategies, the research and development of HPV vaccine and the formulation of immunization plan.

Materials and methods

Study population

~~Samples~~Participants were ~~collected~~ recruited from patients diagnosed according to the Guideline for the Clinical Management of Anogenital Warts in China (2014) [21]~~Diagnosis of Condyloma Acuminatum of China~~, from Shandong Provincial Hospital for Skin Diseases, Shandong Oriental Andrology Hospital, and Jining No.1 People's Hospital from August 2019 to June 2021. All recruited patients had never been vaccinated against HPV. The selection of sentinel hospitals was based on the number of patients diagnosed with CA in the past.

After filling in ~~accepting~~ a questionnaire administered by trained interviewers, each patient underwent ~~biopsy~~sample collection for HPV

typing. ~~Each individual of the patients must volunteer to participate in this study and sign the informed consent.~~ All patients volunteered to participate in this study and signed the informed consent (Minors under the age of 18 had the consent of their guardians). Our study was approved by the ethics committee of school of public health, Shandong University, China (protocol n. 20190320).

Specimen Collection

All patients undergo a clinical examination by a trained clinician at each visit to determine whether there are CA lesions in the cervical, vaginal, vulva, anal and perianal areas in women or in the external genital, anal and perianal sites in men. If the CA lesion is found, the sample will be collected from the site by clinician.~~All samples were collected by uniformly trained clinicians at each clinic visit.~~ Exfoliated cell specimen was collected from the surface of each CA lesion by using a sampling brush. The brush was rotated 3-5 full turns in clockwise direction to ensure acquisition of the exfoliated cells. For each patient, each specimen was independently eluted from the sampling brush with normal saline and stored in the refrigerator at -80°C before detecting HPV genotypes.

HPV genotyping

All HPV DNA extraction, amplification and genotype identification were performed with the HPV genotyping panel (polymerase chain reaction (PCR)-reverse dot blot hybridization method; Yaneng Biosciences,

Shenzhen, China). The HPV genotyping panel can detect 17 high-risk HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73 and 82) and 6 low-risk HPV genotypes (6, 11, 42, 43, 81 and 83). Genomic DNA was extracted from specimens following manufacturer's protocols. The PCR cycling conditions were as follows: initial denaturation at 95°C for 5 min, then 40 cycles at 94°C for 30 s, 42°C for 90 s, 72°C for 30 s, and a final extension at 72°C for 5 min. The HPV type-specific probes are immobilized on nylon membranes for reverse-blot hybridization on, which can detect 23 types of HPV genotypes in a single test. Hybridization was performed and visualized by assessing the protocol according to the manufacturer's instructions. The quality and integrity of the extracted DNA is monitored by the β-actin gene assay. The specimens with known HPV genotypes were used as positive controls and enzyme-free water was used as a negative control.~~HPV tests were performed with the HPV genotyping panel (polymerase chain reaction (PCR)-reverse dot blot hybridization method; Yaneng Biosciences, Shenzhen, China). This multiplex PCR technique can detect 17 high-risk HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73 and 82) and 6 low-risk HPV genotypes (6, 11, 42, 43, 81 and 83). All procedures were carried out following the manufacturer's instructions.~~

Statistical Analysis

Qualitative categorical variables were described as numbers and

percentages. Quantitative variables were described using mean and standard deviation or median and interquartile range (P25- P75), depending on the distribution of the variables. *T*-test was used to compare quantitative variables with a normal distribution, and Mann-Whitney *U*-test was used to compare quantitative variables without normal distribution. Chi-square test or Fisher' exact test was conducted to test hypothesis, as appropriate. All hypothesis tests were two-sided, and *P*-value less than or equal to 0.05 was considered statistically significant. All data were analyzed using SPSS software (version 20.0; IBM Corp.).

Result

CA patients' characteristics

A total of 1185 patients (870 males and 315 females) were included in this study. Shandong Provincial Hospital for Skin Diseases contributed 52.8% of the total participants, whilst 34.4% and 12.8% were from Jining No.1 People's Hospital and Shandong Oriental Andrology Hospital, respectively. The ages ranged from 15 to 88, with a mean of 34.85 years (SD = 13.60 years). The mean age varied slightly between males and females (35.90 vs. 31.94 years; $P < 0.01$). Among recruited patients, 63.7% cases reported having more than one sexual partner. The vast majority of patients who reported their sexual preferences were heterosexual (89.1%), including 745 (70.4%) heterosexual men (men who have sex with women, MSW) and

312 (29.5%) heterosexual women. There were 101 patients who were homosexual, including 99 males (men who had sex with men, MSM) and 2 females. In addition, 26 males and 1 female were bisexual who had sex with both males and females.~~There were 99 men (11.4% of the male patients) who had sex with men (MSM), and 2 women were homosexuals. 26 men and 1 woman who had sex with men and women.~~ Compared with female patients, male patients had more sexual partners ($P < 0.001$). In this study, 85.7% of the patients (86.8% of males and 85.0% of females) themselves claimed that their sexual partners were not infected with HPV. Of the 1185 patients with genital warts, ~~1~~one or more subsequent genital warts had been detected in 411 patients (33.1% of males and 39.0% of females). In addition, there have 32 patients reported other STDs (Fifteen~~15~~ with syphilis, ~~5~~five with genital herpes, ~~6~~six with HIV/AIDS, ~~4~~four with gonorrhea and ~~2~~two with chlamydia infection).

The majority of patients presented warts lesions at one location (53.5%), 34.3%~~The majority of patients stated that only a single body location was infected with warts (53.5%). 34.26%~~ patients presented warts at two different locations, 8.8% at three, and 3.4% at four or more different locations. In females, the CA lesions were often located on the urethral orifice / vaginal introitus (~~62.86~~62.9% of cases), labia majora / labia minora (~~35.87~~35.9%), and perianal region (~~26.35~~26.4%), whereas, for males, the corona sulcus (~~35.98~~36.0%), foreskin (~~26.44~~26.4%) and

perianal region (~~23.56~~23.6%) were the most frequent sites.

~~Table 1. Baseline characteristics of 1185 patients with condyloma acuminatum, by gender~~

~~Characteristics~~	~~Male (n = 870) n (%)~~	~~Female (n= 315) n (%)~~
~~Age~~		
~~< 18~~	~~18 (2.1)~~	~~13 (4.1)~~
~~18-24~~	~~202 (23.2)~~	~~99 (31.4)~~
~~25-29~~	~~120 (13.8)~~	~~38 (12.1)~~
~~30-39~~	~~228 (26.2)~~	~~92 (29.2)~~
~~40-49~~	~~146 (16.8)~~	~~38 (12.1)~~
~~50-59~~	~~93 (10.7)~~	~~28 (8.9)~~
~~≥60~~	~~63 (7.2)~~	~~28 (2.2)~~
~~Marital status~~		
~~Single~~	~~319 (36.7)~~	~~124 (39.4)~~
~~Married~~	~~535 (61.1)~~	~~182 (57.8)~~
~~Widowed/Separated/Divorced~~	~~19 (2.2)~~	~~9 (2.9)~~
~~Education~~		
~~Primary/Middle school~~	~~225 (25.9)~~	~~94 (29.8)~~
~~High school~~	~~209 (24.0)~~	~~49 (15.6)~~
~~University/College~~	~~419 (48.2)~~	~~167 (53.0)~~
~~Postgraduate~~	~~17 (2.0)~~	~~5 (1.6)~~
~~Area of residence at diagnosis~~		
~~Rural area~~	~~263 (30.2)~~	~~96 (30.5)~~
~~City~~	~~607 (69.8)~~	~~219 (69.5)~~
~~Age of first sexual intercourse~~		
~~< 18~~	~~76 (8.7)~~	~~23 (7.3)~~
~~18-21~~	~~431 (49.5)~~	~~155 (49.2)~~
~~≥22~~	~~363 (41.7)~~	~~137 (43.5)~~
~~Type of CA diagnosis~~		
~~Recurrent disease~~	~~288 (33.1)~~	~~123 (39.0)~~
~~New diagnosis~~	~~582 (75.2)~~	~~192 (61.0)~~
~~Condom use~~		
~~No~~	~~108 (12.4)~~	~~34 (10.8)~~
~~Yes, sometimes/rarely~~	~~676 (77.7)~~	~~260 (82.5)~~
~~Yes, always~~	~~86 (9.9)~~	~~21 (6.7)~~
~~Weekly frequency of sexual~~		
~~≤1~~	~~394 (45.6)~~	~~123 (39.0)~~
~~=2~~	~~314 (36.1)~~	~~118 (37.5)~~

~~≥3~~	~~159 (18.3)~~	~~74 (23.5)~~
~~Wash genitalia before sex~~		
~~Yes~~	~~543 (62.4)~~	~~191 (60.6)~~
~~No~~	~~327 (37.6)~~	~~124 (39.4)~~
~~Number of sexual partners~~		
~~=1~~	~~288 (33.1)~~	~~142 (45.1)~~
~~≥2~~	~~582 (66.9)~~	~~173 (54.9)~~

HPV prevalence

A total of 880 samples were collected from 659 males and 221 females with a clinical diagnosis of CA. Shandong Dermatology Hospital collected 407 samples, while Jining No.1 People's Hospital and Shandong Oriental Anorectal Hospital collected 321 and 152 samples, respectively. The HPV DNA was detected in 804 of the 880 CA cases, with a positive rate of 91.4% (90.6% males and 93.7% females).~~In total, 880 samples were collected from 659 males and 221 females with a clinical diagnosis of CA. HPV DNA was detected in 804 of 880 CA cases, yielding an overall prevalence of 91.4% (90.6% males and 93.7% females).~~ Seventy-six~~76~~ samples (from 62 males and 14 females) were excluded from further analysis due to PCR inhibition or insufficient DNA.

Among the 804 patients, 734 (91.3%) patients had at least one LR-HPV infection detected, and 430 (53.5%) patients had HR-HPV infection. In addition, there were 374 (46.5%) patients ~~were~~ only infected with LR-HPV, 70 (8.7%) patients were only infected with HR-HPV, and 360 (44.8%) patients were infected with both LR- HPV and HR-HPV. Compared with

male patients, female patients had a higher rate of HR-HPV infection ($P < 0.001$). Moreover, co-infection of LR-HPV and HR-HPV was more common in female patients ($P < 0.001$). In addition, the prevalence of single HPV genotype infections was 44.2%, while those of multiple HPV genotype coinfections were 55.8%. The total number of HPV genotypes in patients varied from 1 to 12. The frequency of single and multiple infections varied significantly by gender, with single infections being more common among males (47.9% males vs. 33.3% females, $P < 0.001$) and multiple infections more common among females (52.1% males vs. 66.7% females, $P < 0.001$). (Table 2)

Table 2. Distribution of infection types in 804 cases of condyloma acuminatum

	Males (n = 597) [a] n (%)	Females (n= 207) [a] n (%)	*P* value
HR-HPV	293(49.1)	137(66.2)	< 0.001
LR-HPV	543(91.0)	191(92.3)	0.563
LR and HR HPV	239 (40.0)	121 (58.5)	< 0.001
Single infection	286 (47.9)	69 (33.3)	< 0.001
Multiple infections	311 (52.1)	138 (66.7)	< 0.001
HPV6 or 11	515 (86.3)	188 (90.8)	0.088
HPV16 or 18	109 (18.3)	49 (23.7)	0.091
Types of 4-valent vaccines [b]	552 (92.5)	196 (94.7)	0.279
Types of 9-valent vaccines [c]	566 (94.8)	200 (96.6)	0.290

[a] Patients with unqualified HPV results were excluded from the analysis.

[b] The types of 4-valent vaccines conclude HPV 6, 11, 16 or 18.

[c] The types of 9-valent vaccines conclude HPV 6, 11, 16, 18, 31, 33, 45, 52 or 58.

Distribution of specific HPV genotypes

Overall, all 23 different HPV genotypes were identified in males and

females, with a proportion ranging from 1.5% to 57.8% (12/804 to 465/804). The most frequently encountered HPV genotypes were HPV 6 (57.8%), HPV 11 (37.2%), HPV 16 (13.7%), HPV 42 (10.3%) and HPV 52 (8.8%) by decreasing order of frequency (Figure 1).

As it was shown, ~~HPV 6 (57.8%), HPV 11 (37.2%) and HPV 42 (10.3%) were the three most common LR-HPV genotypes in our study population.~~ HPV 6 was the most common HPV genotype detected in both males (58.1%) and females (57.0%). HPV 6/11 (alone or in coinfection with other genotypes) were detected in 87.4% patients and there was no significant difference among males and females. In addition, there was no significant difference in the distribution of all detective low-risk genotypes between males and females. The most common HR-HPV genotypes were HPV 16 (13.7%), HPV 52 (8.8%) and HPV 51 (8.1%). HPV 16/18 (alone or in coinfection with other genotypes) were observed in 19.7% patients. In the HR-HPV genotypes, the frequency of HPV 45, HPV 56 and HPV 58 were significantly higher in women than in men ($P < 0.05$). Among the patients coinfected with LR-HPV and HR-HPV, the most infection mode is HPV6+HPV16 (5.6%). Different frequency of other HPV genotypes in different genders were shown in Table 3.

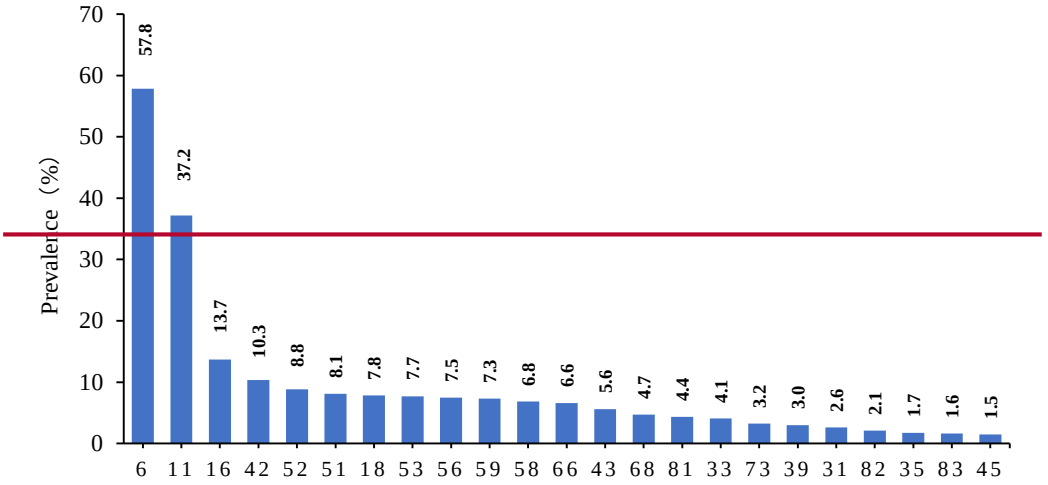

Figure 1 Prevalence of specific human papillomavirus (HPV) infection by decreasing order of frequency. Patients with unqualified HPV results were excluded from the analysis.

Table 3. Prevalence of specific human papillomavirus (HPV) genotypes among 804 cases of condyloma acuminatum, by decreasing order of frequency.

HPV genotypes	Males (n = 597)[a] n (%)	Females (n= 207)[a] n (%)	*P* value
LR genotypes			
HPV6	347 (58.1)	118 (57.0)	0.779
HPV11	214 (35.8)	85 (41.4)	0.181
HPV42	64 (10.7)	19 (9.2)	0.530
HPV43	37 (6.2)	8 (3.9)	0.208
HPV81	23 (3.9)	12 (5.8)	0.237
HPV83	10 (1.7)	3 (1.4)	0.824
HR genotypes			
HPV16	77 (12.9)	33 (15.9)	0.272
HPV52	51 (8.5)	20 (9.7)	0.625
HPV51	43 (7.2)	22 (10.6)	0.119
HPV18	43 (7.2)	20 (9.7)	0.257
HPV53	44 (7.4)	18 (8.7)	0.538
HPV56	36 (6.0)	24 (11.6)	0.009
HPV59	44 (7.4)	15 (7.2)	0.953
HPV58	34 (5.7)	21 (10.1)	0.029
HPV66	36 (6.0)	17 (8.2)	0.276
HPV68	24 (4.0)	14 (6.8)	0.109
HPV33	20 (3.4)	13 (6.3)	0.067
HPV73	23 (3.9)	3 (1.4)	0.092
HPV39	17 (2.8)	7 (3.4)	0.697
HPV31	16 (2.7)	5 (2.4)	0.837
HPV82	12 (2.0)	5 (2.4)	0.727

HPV35	8 (1.3)	6 (2.9)	0.140
HPV45	5 (0.8)	7 (3.4)	0.009

[a] Patients with unqualified HPV results were excluded from the analysis.

Discussion

As we all know, CA is one of the most common sexually transmitted infections diseases (STIsSTDs) caused by HPV infection, and most sexually active persons are usually exposed to HPV during their lifetime [1922]. In this study based on surveys in sentinel hospitals, we reported the prevalent characteristics of CA and distribution of HPV genotypes in patients with CA in Shandong Province, China. In our study, a total of 1185 CA patients were recruited. The recruited patients were mainly males. This may be due to the differences in the anatomical structure of males and females. Compared with males, females need to undergo a professional gynecological examination in the hospital to find out whether they have CA. The average age of CA patients recruited in this study was 34.85 years. Compared with men, women were younger. These results are consistent with a previous Chinese multi-center study of genital wart conducted in 2013 including 1,005 cases from eighteen hospitals in seven geographical regions of China [2023]. In this study, most patients were in the sexually active stage aged 20 to 40. These patients had risky sexual behaviors such as multiple sexual partners and irregular condom use. Among them, 32 cases also suffered from other sexually transmitted diseases. These are all risk factors that increase HPV infection [2124, 2225]. In addition, 34.7%

of the patients in this study were recurrences.

Among the 880 patients who underwent HPV testing, the overall HPV ~~prevalence~~test positivity rate was ~~91.36~~91.4%, which shows that HPV is the pathogen that causes CA. There were 8.6% of the patients could not be included in the analysis due to insufficient cellular DNA content. Previous~~Some~~ studies have found that 16~26% of the samples will be negative regardless of the type of cell brush sampling [~~23~~26, ~~24~~27]. Compared with other studies, the lower negative rate of our study reflects the higher quality of sample collection after unified training. In the analysis of specific HPV types, it was shown that the most common genotypes in Shandong Province, China were HPV6, HPV11, HPV16 and HPV42. Compared with other regions in China, HPV42 had a higher prevalence in Shandong province and had become one of the common genotypes of HPV in CA patients. Previous studies have found that the distribution of HPV genotypes varies from region to region. Although the risk of diseases related to nonvaccine HPV genotypes is theoretically still low, the actual situation still needs to be monitored to deal with the disease burden caused by high prevalence of nonvaccine HPV genotypes. Furthermore, the distribution of HPV6, HPV11 and HPV16 were consistent with the results of studies in other regions of China [~~20~~23, ~~25~~28, ~~26~~29].

In this study, males were mainly infected with low-risk HPV, while females were mainly multiple infected with high-risk and low-risk HPV. Most

patients, both male and female, were infected with more than one different HPV genotype. According to our results, HPV6 and 11 (alone or coinfection with other genotypes) were detected in 87.4% of patients, but most of these patients also had high-risk HPV infection. In this study, 53.5% of patients with CA were infected with high-risk HPV. Among male patients, 49.1% ~~of male patients~~ were infected with at least one high-risk type of HPV. Some studies have shown that male penile cancer and anal cancer are associated with high-risk HPV infection [~~27~~30]. Compared with male patients, female patients had a higher infection rate of high-risk HPV (66.2% vs. 49.1%), indicating that women had a higher burden of high-risk HPV infection. Persistent high-risk HPV infection can lead to precancerous lesions and cancers of the cervix, vulva, vagina, and anus in women, so it is very important to prevent the occurrence of these lesions [~~28~~31]. Some studies find that high HPV vaccination coverage significantly reduced the prevalence of CA, HPV infection and cervical intraepithelial neoplasia grade 2+ (CIN2+) [~~29~~32, ~~30~~33]. In addition, when formulating policies to prevent HPV infection in women, it should be considered that men also have a higher infection rate of high-risk HPV. Some studies have found that men HPV multiple infection is very common, and the high-risk HPV infection rate is the same as that of women [~~31~~34-~~33~~36]. There is a higher risk of cross-infection between men and women [~~34~~37, ~~35~~38]. HPV vaccination to men can effectively reduce related HPV infection rates and

HPV-related cancer incidence [~~36~~39, ~~37~~40].

Nowadays, four prophylactic HPV vaccines are available in China, including the HPV bivalent vaccine (Cervarix®; Cecolin®) to protect against HPV16 and 18; the HPV quadrivalent vaccine (Gardasil®) to protect against HPV 6, 11, 16 and 18; the HPV nonavalent vaccine (Gardasil®9) to protect against HPV 6, 11, 16, 18, 31, 33, 45, 52 and 58 [~~38~~41, ~~39~~42]. At present, there is no HPV vaccination programs implemented in China, which may be related to the insufficient data of HPV epidemiological survey. However, the distribution of HPV genotypes varies from region to region [~~40~~43]. Although vaccines can provide protection for people, it is important to study the prevalence and distribution of HPV genotypes in different regions before implementing the HPV vaccination plan, because understanding the distribution of HPV genotypes will help to choose the best vaccine for HPV protection in each region [44]. In this study, HPV6 and HPV11 were the causes of CA in the majority of patients (87.4%), and they were included in both the quadrivalent and nonavalent vaccines. Considering the economic benefits and the insufficient supply of 9-valent HPV vaccine in China, the 4-valent HPV vaccine may be more suitable for the prevention of CA in Shandong Province, China. Furthermore ,HPV42, as one of the genotypes with higher prevalence in this study, has not been included in any licensed HPV vaccines. Previous studies have found that among people vaccinated with

the quadrivalent HPV vaccine, the infection rate of nonvaccine HPV genotypes is remaining flat or higher [~~41~~45]. Therefore, it is necessary to develop a broader spectrum of HPV vaccines to reduce the disease burden caused by HPV infection [~~42~~46].

In conclusion, CA cases are mainly infected with low-risk HPV, but there are more high-risk HPV infections in both males and females, which is a problem that cannot be ignored in clinical treatment. It is necessary to monitor the HPV genotype of CA patients, because high-risk HPV infection is the main risk factor for cancer. For the general public, HPV typing and HPV vaccination will help to prevent CA or HPV-related cancer and reduce the risk of disease. Additionally, nonvaccine HPV genotypes also need to be monitored for their prevalence by regular HPV typing. The results of our study ~~provide~~make a contribution to the epidemiology of HPV in Chinese CA patients with improving the understanding of existing HPV genotypes. Furthermore, these will have a positive impact on the adjustment of CA prevention and treatment strategies, the research and development of HPV vaccines and the formulation of immunization programs.

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Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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Ethics and dissemination

Our study was approved by the ethics committee of School of Public Health, Shandong University, China (protocol n. 20190320). All patients volunteered to participate in this study and signed the informed consent (Minors under the age of 18 had the consent of their guardians).

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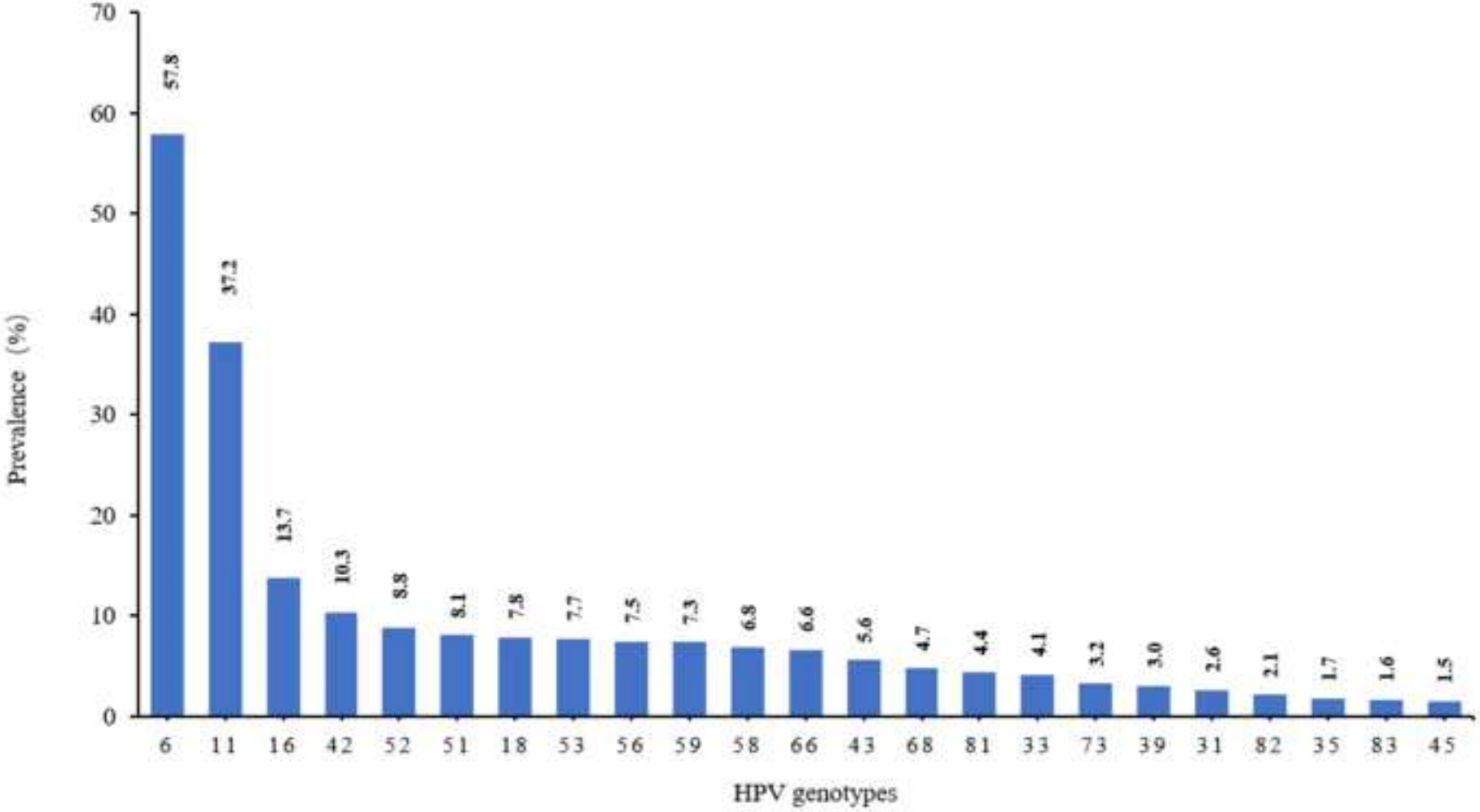

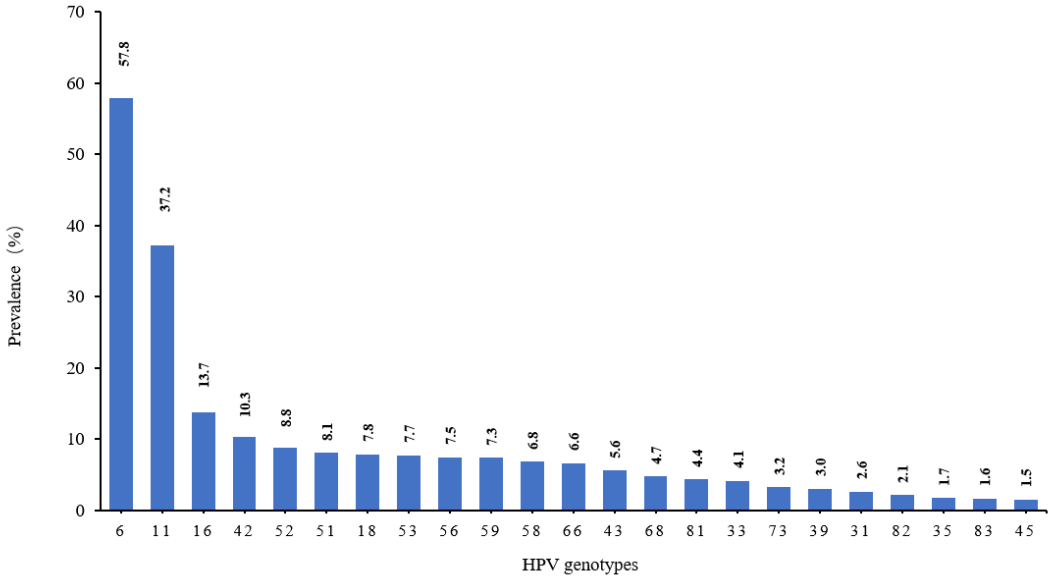

Figure 1 Prevalence of specific human papillomavirus (HPV) infection by decreasing order of frequency.

*Patients with unqualified HPV results were excluded from the analysis.

Table 1. Baseline characteristics of with condyloma acuminatum patients, by gender

Characteristics	Male (n = 870) n (%)	Female (n= 315) n (%)
Age		
< 18	18 (2.1)	13 (4.1)
18-24	202 (23.2)	99 (31.4)
25-29	120 (13.8)	38 (12.1)
30-39	228 (26.2)	92 (29.2)
40-49	146 (16.8)	38 (12.1)
50-59	93 (10.7)	28 (8.9)
≥60	63 (7.2)	7 (2.2)
Marital status		
Single	319 (36.7)	124 (39.3)
Married	532 (61.1)	182 (57.8)
Widowed/Separated/Divorced	19 (2.2)	9 (2.9)
Education		
Primary/Middle school	225 (25.9)	94 (29.8)
High school	209 (24.0)	49 (15.6)
University/College	419 (48.2)	167 (53.0)
Postgraduate	17 (1.9)	5 (1.6)
Area of residence at diagnosis		
Rural area	263 (30.2)	96 (30.5)
City	607 (69.8)	219 (69.5)
Age of first sexual intercourse		
< 18	76 (8.7)	23 (7.3)
18-21	431 (49.6)	155 (49.2)
≥22	363 (41.7)	137 (43.5)
Type of CA diagnosis		
Recurrent disease	288 (33.1)	123 (39.0)
New diagnosis	582 (66.9)	192 (61.0)
Condom use		
No	108 (12.4)	34 (10.8)
Yes, sometimes/rarely	676 (77.7)	260 (82.5)
Yes, always	86 (9.9)	21 (6.7)
Weekly frequency of sex		
≤1	397 (45.6)	123 (39.0)
=2	314 (36.1)	118 (37.5)
≥3	159 (18.3)	74 (23.5)
Wash genitalia before sex		

Yes	543 (62.4)	191 (60.6)
No	327 (37.6)	124 (39.4)
Number of sexual partners		
=1	288 (33.1)	142 (45.1)
≥2	582 (66.9)	173 (54.9)

Table 2. Distribution of infection types in condyloma acuminatum patients, by gender

	Males (n = 597) [a] n (%)	Females (n= 207) [a] n (%)	*P* value
HR-HPV	293(49.1)	137(66.2)	< 0.001
LR-HPV	543(91.0)	191(92.3)	0.563
Only HR-HPV	54 (9.0)	16 (7.7)	0.563
Only LR-HPV	304 (50.9)	70 (33.8)	< 0.001
LR and HR HPV	239 (40.0)	121 (58.5)	< 0.001
Single infection	286 (47.9)	69 (33.3)	< 0.001
Multiple infections	311 (52.1)	138 (66.7)	< 0.001
HPV6 or 11	515 (86.3)	188 (90.8)	0.088
HPV16 or 18	109 (18.3)	49 (23.7)	0.091
Types of 4-valent vaccines [b]	552 (92.5)	196 (94.7)	0.279
Types of 9-valent vaccines [c]	566 (94.8)	200 (96.6)	0.290

[a] Patients with unqualified HPV results were excluded from the analysis.

[b] The types of 4-valent vaccines conclude HPV 6, 11, 16 or 18.

[c] The types of 9-valent vaccines conclude HPV 6, 11, 16, 18, 31, 33, 45, 52 or 58.

Table 3. Prevalence of specific human papillomavirus (HPV) genotypes in condyloma acuminatum patients, by decreasing order of frequency.

HPV genotypes	Males (n = 597) [a] n (%)	Females (n= 207) [a] n (%)	*P* value
LR genotypes			
HPV6	347 (58.1)	118 (57.0)	0.779
HPV11	214 (35.8)	85 (41.4)	0.181
HPV42	64 (10.7)	19 (9.2)	0.530
HPV43	37 (6.2)	8 (3.9)	0.208
HPV81	23 (3.9)	12 (5.8)	0.237
HPV83	10 (1.7)	3 (1.4)	0.824
HR genotypes			
HPV16	77 (12.9)	33 (15.9)	0.272
HPV52	51 (8.5)	20 (9.7)	0.625
HPV51	43 (7.2)	22 (10.6)	0.119
HPV18	43 (7.2)	20 (9.7)	0.257
HPV53	44 (7.4)	18 (8.7)	0.538
HPV56	36 (6.0)	24 (11.6)	0.009
HPV59	44 (7.4)	15 (7.2)	0.953
HPV58	34 (5.7)	21 (10.1)	0.029
HPV66	36 (6.0)	17 (8.2)	0.276
HPV68	24 (4.0)	14 (6.8)	0.109
HPV33	20 (3.4)	13 (6.3)	0.067
HPV73	23 (3.9)	3 (1.4)	0.092
HPV39	17 (2.8)	7 (3.4)	0.697
HPV31	16 (2.7)	5 (2.4)	0.837
HPV82	12 (2.0)	5 (2.4)	0.727
HPV35	8 (1.3)	6 (2.9)	0.140
HPV45	5 (0.8)	7 (3.4)	0.009

[a] Patients with unqualified HPV results were excluded from the analysis.

Review II

Close

KHVI-2022-0554R1
"Epidemiology of Human Papillomavirus on Condyloma Acuminatum in Shandong Province, China"
Revision 1

Gondo Mastutik (Reviewer 2)

Reviewer Recommendation Term:	Accept with minor revision
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Comments to Editor:

Dear Professor Ronald Ellis Ph.D. MBA
In general, the authors have edited and revised well. But there are still some minor corrections. I am attaching a manuscript that I have highlighted to give to the author.
Thank you very much for the opportunity given to me to review the article. I suggest accepting with a minor correction.

Best Regard,
Gondo Mastutik

Comments to Author:

Dear authors,

Thank you very much for revising and editing this manuscript so well. However, it is our duty to review this article. Please find the results of the review in the manuscript that we have given a high light yellow (as attactment).
1. Line 67 & 68. On the other hand, the treatment fee is expensive ("on the other hand" in this sentence, it would be better changed to " in addition").
2. Please mention Table in the main tex. (Table 1 has not been mentioned in the main text).
3. Line 307. Province, China. Furthermore, HPV42, as one of the genotypes with higher. Please, delete space between Furthermore and ", ". To be "Furthermore,"
4. Line 192. were infected with both LR- HPV and HR-HPV. Compared with male. Please delete space between LR- and HPV, it would be "LR-HPV".
5. In all sentences, it should be consistent in writing the HPV genotype. For example HPV space 6 (HPV 6) or HPV6.
Please choose one typing type. Please check all sentences. Example line 50, 253, 254, etc.

6. In the line 71. It was mentioned that "as high-risk (HR) or low-risk (LR)", but in the line 264, 265, 269, etc, it mentioned with "high-risk and low-risk HPV".
Please be consistent to use HR or LR after making abbreviations.
7. Please be consistent in mentioning reference sources. After the "point", then mention the reference source number OR mention the reference source number first, then "point".
Example line 69: burden to patients and their families. [5-7] OR line 72, 73, 74, 75, 76, etc. least 40 genotypes are associated with infection in the anogenital region [8-10].

Thank you very much and good luck.

Attachments:

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Epidemiology of Human Papillomavirus on Condyloma Acuminatum in Shandong Province，China

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Abstract

Condyloma acuminatum (CA) is a sexually transmitted disease (STD) caused by human papillomavirus (HPV) infection. It is important to study the prevalence and distribution of HPV genotypes before implementing HPV vaccination program. Therefore, the aim of this study was to evaluate the epidemiological characteristics of CA cases and the distribution of HPV genotypes in Shandong Province, China. One-to-one questionnaire surveys were conducted on all patients diagnosed with CA in sentinel hospitals from Shandong Province, China. HPV genotypes were determined using the polymerase chain reaction (PCR)-reverse dot blot hybridization method. The study enrolled 1185 patients (870 males and 315 females) and found that CA patients are mainly males and sexually active people between the ages of 20 and 40. Recurrence occurred in 34.7% patients. Among the 880 CA patients who underwent HPV typing, the HPV test positivity rate was 91.4%. In these cases, low-risk HPV genotypes infection was predominant,

with an infection rate of 91.3%, while high-risk HPV genotypes were found in 53.5% patients. The most frequent HPV genotypes encountered were HPV 6 (57.8%), HPV 11 (37.2%), HPV 16 (13.7%) and HPV 42 (10.3%). HPV 6 and/or HPV 11 are the main infections in all patients, and more than half of the patients are coinfected with high-risk HPV. However, unlike other regions, HPV42 has a higher prevalence rate among CA patients in Shandong Province and is a nonvaccine HPV genotype. Therefore, regular HPV typing helps to understand the characteristics of specific genotypes and the choice of the best type for vaccine coverage.

Keywords: condyloma acuminatum; human papillomavirus; epidemiology; HPV genotypes

Introduction

Condyloma acuminatum (CA), also known as genital warts (GW), is a verrucous hyperplasia of squamous epithelium in the anogenital region caused by human papillomavirus (HPV) infection, and it is one of the most common sexually transmitted diseases (STDs) [1, 2]. The CA is the most common benign lesions of the anogenital region, but it can be difficult to treat and easy to recur, which required repeated treatment [3]. Studies have shown that approximately 48.5% of CA patients will experience recurrence [4]. The occurrence of CA reduces the quality of life of patients. On the one hand, the affected area of CA is private and the treatment process is

painful, which brings serious psychological pressure to patients. On the other hand, the treatment fee is expensive, which brings heavy financial burden to patients and their families. [5-7].

At present, more than 200 HPV genotypes have been identified and classified as high-risk (HR) or low-risk (LR), based on oncogenic risk. At least 40 genotypes are associated with infection in the anogenital region [8-10]. HPV 6 and 11 are associated with approximately 90% of CA, whereas 70% of cervical cancers are caused by HPV16 and 18[11]. Patients with CA are often accompanied by HR-HPV infection [12-15]. Persistent HR-HPV infection is the main pathogenic factor of cervical intraepithelial neoplasia and cervical cancer in women [16,17].

There is reliable evidence that HPV vaccination has a good preventive effect against HPV infection in uninfected individuals, subsequent CA and HPV-attributable cancers, thereby greatly reducing the burden of HPV-related diseases in patients [18, 19]. However, the HPV vaccines-targeted types are limited, and the HPV genotypes varies among different regions and populations [20].

At present, the HPV infection and epidemiological characteristics of CA patients in China are rare and incomplete. Therefore, in this survey, the evaluation of the epidemic characteristics and HPV genotype distribution of CA cases in Shandong Province, China will contribute to the adjustment of CA prevention and control strategies, the research and development of

HPV vaccine and the formulation of immunization plan.

Materials and methods

Study population

Participants were recruited from patients diagnosed according to the Guideline for the Clinical Management of Anogenital Warts in China (2014) [21], from Shandong Provincial Hospital for Skin Diseases, Shandong Oriental Andrology Hospital, and Jining No.1 People's Hospital from August 2019 to June 2021. All recruited patients had never been vaccinated against HPV. The selection of sentinel hospitals was based on the number of patients diagnosed with CA in the past.

After filling in a questionnaire administered by trained interviewers, each patient underwent sample collection for HPV typing. All patients volunteered to participate in this study and signed the informed consent (Minors under the age of 18 had the consent of their guardians). Our study was approved by the ethics committee of school of public health, Shandong University, China (protocol n. 20190320).

Specimen Collection

All patients undergo a clinical examination by a trained clinician at each visit to determine whether there are CA lesions in the cervical, vaginal, vulva, anal and perianal areas in women or in the external genital, anal and perianal sites in men. If the CA lesion is found, the sample will be collected

from the site by clinician. Exfoliated cell specimen was collected from the surface of each CA lesion by using a sampling brush. The brush was rotated 3-5 full turns in clockwise direction to ensure acquisition of the exfoliated cells. For each patient, each specimen was independently eluted from the sampling brush with normal saline and stored in the refrigerator at -80°C before detecting HPV genotypes.

HPV genotyping

All HPV DNA extraction, amplification and genotype identification were performed with the HPV genotyping panel (polymerase chain reaction (PCR)-reverse dot blot hybridization method; Yaneng Biosciences, Shenzhen, China). The HPV genotyping panel can detect 17 high-risk HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73 and 82) and 6 low-risk HPV genotypes (6, 11, 42, 43, 81 and 83). Genomic DNA was extracted from specimens following manufacturer's protocols. The PCR cycling conditions were as follows: initial denaturation at 95°C for 5 min, then 40 cycles at 94°C for 30 s, 42°C for 90 s, 72°C for 30 s, and a final extension at 72°C for 5 min. The HPV type-specific probes are immobilized on nylon membranes for reverse-blot hybridization on, which can detect 23 types of HPV genotypes in a single test. Hybridization was performed and visualized by assessing the protocol according to the manufacturer's instructions. The quality and integrity of the extracted DNA is monitored by the β-actin gene assay. The specimens with known HPV

genotypes were used as positive controls and enzyme-free water was used as a negative control.

Statistical Analysis

Qualitative categorical variables were described as numbers and percentages. Quantitative variables were described using mean and standard deviation or median and interquartile range (P25- P75), depending on the distribution of the variables. *T*-test was used to compare quantitative variables with a normal distribution, and Mann-Whitney *U*-test was used to compare quantitative variables without normal distribution. Chi-square test or Fisher' exact test was conducted to test hypothesis, as appropriate. All hypothesis tests were two-sided, and *P*-value less than or equal to 0.05 was considered statistically significant. All data were analyzed using SPSS software (version 20.0; IBM Corp.).

Result

CA patients' characteristics

A total of 1185 patients (870 males and 315 females) were included in this study. Shandong Provincial Hospital for Skin Diseases contributed 52.8% of the total participants, whilst 34.4% and 12.8% were from Jining No.1 People's Hospital and Shandong Oriental Andrology Hospital, respectively. The ages ranged from 15 to 88, with a mean of 34.85 years (SD = 13.60 years). The mean age varied slightly between males and females (35.90 vs.

31.94 years; $P < 0.01$). Among recruited patients, 63.7% cases reported having more than one sexual partner. The vast majority of patients who reported their sexual preferences were heterosexual (89.1%), including 745 (70.4%) heterosexual men (men who have sex with women, MSW) and 312 (29.5%) heterosexual women. There were 101 patients who were homosexual, including 99 males (men who had sex with men, MSM) and 2 females. In addition, 26 males and 1 female were bisexual who had sex with both males and females. Compared with female patients, male patients had more sexual partners ($P < 0.001$). In this study, 85.7% of the patients (86.8% of males and 85.0% of females) themselves claimed that their sexual partners were not infected with HPV.

Of the 1185 patients with genital warts, one or more subsequent genital warts had been detected in 411 patients (33.1% of males and 39.0% of females). In addition, there have 32 patients reported other STDs (Fifteen with syphilis, five with genital herpes, six with HIV/AIDS, four with gonorrhea and two with chlamydia infection).

The majority of patients presented warts lesions at one location (53.5%), 34.3% patients presented warts at two different locations, 8.8% at three, and 3.4% at four or more different locations. In females, the CA lesions were often located on the urethral orifice / vaginal introitus (62.9% of cases), labia majora / labia minora (35.9%), and perianal region (26.4%), whereas, for males, the corona sulcus (36.0%), foreskin (26.4%) and

perianal region (23.6%) were the most frequent sites.

HPV prevalence

A total of 880 samples were collected from 659 males and 221 females with a clinical diagnosis of CA. Shandong Dermatology Hospital collected 407 samples, while Jining No.1 People's Hospital and Shandong Oriental Anorectal Hospital collected 321 and 152 samples, respectively. The HPV DNA was detected in 804 of the 880 CA cases, with a positive rate of 91.4% (90.6% males and 93.7% females). Seventy-six samples (from 62 males and 14 females) were excluded from further analysis due to PCR inhibition or insufficient DNA.

Among the 804 patients, 734 (91.3%) patients had at least one LR-HPV infection detected, and 430 (53.5%) patients had HR-HPV infection. In addition, there were 374 (46.5%) patients only infected with LR-HPV, 70 (8.7%) patients were only infected with HR-HPV, and 360 (44.8%) patients were infected with both LR- HPV and HR-HPV. Compared with male patients, female patients had a higher rate of HR-HPV infection ($P < 0.001$). Moreover, co-infection of LR-HPV and HR-HPV was more common in female patients ($P < 0.001$). In addition, the prevalence of single HPV genotype infections was 44.2%, while those of multiple HPV genotype coinfections were 55.8%. The total number of HPV genotypes in patients varied from 1 to 12. The frequency of single and multiple infections varied

significantly by gender, with single infections being more common among males (47.9% males vs. 33.3% females, $P < 0.001$) and multiple infections more common among females (52.1% males vs. 66.7% females, $P < 0.001$). (Table 2)

Distribution of specific HPV genotypes

Overall, all 23 different HPV genotypes were identified in males and females, with a proportion ranging from 1.5% to 57.8% (12/804 to 465/804). The most frequently encountered HPV genotypes were HPV 6 (57.8%), HPV 11 (37.2%), HPV 16 (13.7%), HPV 42 (10.3%) and HPV 52 (8.8%) by decreasing order of frequency. (Figure 1)

As it was shown, HPV 6 was the most common HPV genotype detected in both males (58.1%) and females (57.0%). HPV 6/11 (alone or in coinfection with other genotypes) were detected in 87.4% patients and there was no significant difference among males and females. In addition, there was no significant difference in the distribution of all detective low-risk genotypes between males and females. The most common HR-HPV genotypes were HPV 16 (13.7%), HPV 52 (8.8%) and HPV 51 (8.1%). HPV 16/18 (alone or in coinfection with other genotypes) were observed in 19.7% patients. In the HR-HPV genotypes, the frequency of HPV 45, HPV 56 and HPV 58 were significantly higher in women than in men ($P < 0.05$). Among the patients coinfected with LR-HPV and HR-HPV, the

most infection mode is HPV6+HPV16 (5.6%). Different frequency of other HPV genotypes in different genders were shown in Table 3.

Discussion

As we all know, CA is one of the most common sexually transmitted diseases (STDs) caused by HPV infection, and most sexually active persons are usually exposed to HPV during their lifetime [22]. In this study based on surveys in sentinel hospitals, we reported the prevalent characteristics of CA and distribution of HPV genotypes in patients with CA in Shandong Province, China. In our study, a total of 1185 CA patients were recruited. The recruited patients were mainly males. This may be due to the differences in the anatomical structure of males and females. Compared with males, females need to undergo a professional gynecological examination in the hospital to find out whether they have CA. The average age of CA patients recruited in this study was 34.85 years. Compared with men, women were younger. These results are consistent with a previous Chinese multi-center study of genital wart conducted in 2013 including 1,005 cases from eighteen hospitals in seven geographical regions of China [23]. In this study, most patients were in the sexually active stage aged 20 to 40. These patients had risky sexual behaviors such as multiple sexual partners and irregular condom use. Among them, 32 cases also suffered from other sexually transmitted diseases. These are all

risk factors that increase HPV infection [24, 25]. In addition, 34.7% of the patients in this study were recurrences.

Among the 880 patients who underwent HPV testing, the overall HPV test positivity rate was 91.4%, which shows that HPV is the pathogen that causes CA. There were 8.6% of the patients could not be included in the analysis due to insufficient cellular DNA content. Previous studies have found that 16~26% of the samples will be negative regardless of the type of cell brush sampling [26, 27]. Compared with other studies, the lower negative rate of our study reflects the higher quality of sample collection after unified training. In the analysis of specific HPV types, it was shown that the most common genotypes in Shandong Province, China were HPV6, HPV11, HPV16 and HPV42. Compared with other regions in China, HPV42 had a higher prevalence in Shandong province and had become one of the common genotypes of HPV in CA patients. Previous studies have found that the distribution of HPV genotypes varies from region to region. Although the risk of diseases related to nonvaccine HPV genotypes is theoretically still low, the actual situation still needs to be monitored to deal with the disease burden caused by high prevalence of nonvaccine HPV genotypes. Furthermore, the distribution of HPV6, HPV11 and HPV16 were consistent with the results of studies in other regions of China [23, 28, 29].

In this study, males were mainly infected with low-risk HPV, while females

were mainly multiple infected with high-risk and low-risk HPV. Most patients, both male and female, were infected with more than one different HPV genotype. According to our results, HPV6 and 11 (alone or coinfection with other genotypes) were detected in 87.4% of patients, but most of these patients also had high-risk HPV infection. In this study, 53.5% of patients with CA were infected with high-risk HPV. Among male patients, 49.1% were infected with at least one high-risk type of HPV. Some studies have shown that male penile cancer and anal cancer are associated with high-risk HPV infection [30]. Compared with male patients, female patients had a higher infection rate of high-risk HPV (66.2% vs. 49.1%), indicating that women had a higher burden of high-risk HPV infection. Persistent high-risk HPV infection can lead to precancerous lesions and cancers of the cervix, vulva, vagina, and anus in women, so it is very important to prevent the occurrence of these lesions [31]. Some studies find that high HPV vaccination coverage significantly reduced the prevalence of CA, HPV infection and cervical intraepithelial neoplasia grade 2+ (CIN2+) [32, 33]. In addition, when formulating policies to prevent HPV infection in women, it should be considered that men also have a higher infection rate of high-risk HPV. Some studies have found that men HPV multiple infection is very common, and the high-risk HPV infection rate is the same as that of women [34-36]. There is a higher risk of cross-infection between men and women [37, 38]. HPV vaccination to

men can effectively reduce related HPV infection rates and HPV-related cancer incidence [39, 40].

Nowadays, four prophylactic HPV vaccines are available in China, including the HPV bivalent vaccine (Cervarix®; Cecolin®) to protect against HPV16 and 18; the HPV quadrivalent vaccine (Gardasil®) to protect against HPV 6, 11, 16 and 18; the HPV nonavalent vaccine (Gardasil®9) to protect against HPV 6, 11, 16, 18, 31, 33, 45, 52 and 58 [41, 42]. At present, there is no HPV vaccination programs implemented in China, which may be related to the insufficient data of HPV epidemiological survey. However, the distribution of HPV genotypes varies from region to region [43]. Although vaccines can provide protection for people, it is important to study the prevalence and distribution of HPV genotypes in different regions before implementing the HPV vaccination plan, because understanding the distribution of HPV genotypes will help to choose the best vaccine for HPV protection in each region [44]. In this study, HPV6 and HPV11 were the causes of CA in the majority of patients (87.4%), and they were included in both the quadrivalent and nonavalent vaccines. Considering the economic benefits and the insufficient supply of 9-valent HPV vaccine in China, the 4-valent HPV vaccine may be more suitable for the prevention of CA in Shandong Province, China. Furthermore ,HPV42, as one of the genotypes with higher prevalence in this study, has not been included in any licensed HPV

vaccines. Previous studies have found that among people vaccinated with the quadrivalent HPV vaccine, the infection rate of nonvaccine HPV genotypes is remaining flat or higher [45]. Therefore, it is necessary to develop a broader spectrum of HPV vaccines to reduce the disease burden caused by HPV infection [46].

In conclusion, CA cases are mainly infected with low-risk HPV, but there are more high-risk HPV infections in both males and females, which is a problem that cannot be ignored in clinical treatment. It is necessary to monitor the HPV genotype of CA patients, because high-risk HPV infection is the main risk factor for cancer. For the general public, HPV typing and HPV vaccination will help to prevent CA or HPV-related cancer and reduce the risk of disease. Additionally, nonvaccine HPV genotypes also need to be monitored for their prevalence by regular HPV typing. The results of our study make a contribution to the epidemiology of HPV in Chinese CA patients with improving the understanding of existing HPV genotypes. Furthermore, these will have a positive impact on the adjustment of CA prevention and treatment strategies, the research and development of HPV vaccines and the formulation of immunization programs.

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Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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Ethics and dissemination

Our study was approved by the ethics committee of School of Public Health, Shandong University, China (protocol n. 20190320). All patients volunteered to participate in this study and signed the informed consent (Minors under the age of 18 had the consent of their guardians).

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RESEARCH ARTICLE

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Epidemiology of human papillomavirus on condyloma acuminatum in Shandong Province, China

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ABSTRACT

Condyloma acuminatum (CA) is a sexually transmitted disease (STD) caused by human papillomavirus (HPV) infection. It is important to study the prevalence and distribution of HPV genotypes before implementing the HPV vaccination program. Therefore, the aim of this study was to evaluate the epidemiological characteristics of CA cases and the distribution of HPV genotypes in Shandong Province, China. One-to-one questionnaire surveys were conducted on all patients diagnosed with CA in sentinel hospitals from Shandong Province, China. HPV genotypes were determined using the polymerase chain reaction (PCR)-reverse dot blot hybridization method. The study enrolled 1185 patients (870 males and 315 females) and found that CA patients are mainly males and sexually active people between the ages of 20 and 40. Recurrence occurred in 34.7% patients. Among the 880 CA patients who underwent HPV typing, the HPV test positivity rate was 91.4%. In these cases, low-risk (LR) HPV infection was predominant, with an infection rate of 91.3%, while high-risk (HR) HPV genotypes were found in 53.5% patients. The most frequent HPV genotypes encountered were HPV6 (57.8%), HPV11 (37.2%), HPV16 (13.7%), and HPV42 (10.3%). HPV6 and/or HPV11 are the main infections in all patients, and more than half of the patients are coinfected with HR-HPV. However, unlike other regions, HPV42 has a higher prevalence rate among CA patients in Shandong Province and is a nonvaccine HPV genotype. Therefore, regular HPV typing helps to understand the characteristics of specific genotypes and the choice of the best type for vaccine coverage.

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Condyloma acuminatum; human papillomavirus; epidemiology; HPV genotypes

Introduction

Condyloma acuminatum (CA), also known as genital warts (GW), is a verrucous hyperplasia of squamous epithelium in the anogenital region caused by human papillomavirus (HPV) infection, and it is one of the most common sexually transmitted diseases (STDs).[1,2] The CA is the most common benign lesions of the anogenital region, but it can be difficult to treat and easy to recur, which required repeated treatment.[3] Studies have shown that approximately 48.5% of CA patients will experience recurrence.[4] The occurrence of CA reduces the quality of life of patients. Above all, the affected area of CA is private and the treatment process is painful, which brings serious psychological pressure to patients. In addition, the treatment fee is expensive, which brings heavy financial burden to patients and their families.[5–7]

At present, more than 200 HPV genotypes have been identified and classified as high-risk (HR) or low-risk (LR), based on oncogenic risk. At least 40 genotypes are associated with infection in the anogenital region.[8–10] HPV6 and 11 are associated with approximately 90% of CA, whereas 70% of the cervical cancers are caused by HPV16 and 18.[11] Patients with CA are often accompanied by HR-HPV infection.[12–15] Persistent HR-HPV infection is the main pathogenic factor of cervical intraepithelial neoplasia and cervical cancer in women.[16,17]

There is reliable evidence that HPV vaccination has a good preventive effect against HPV infection in uninfected individuals and subsequent CA and HPV-attributable cancers, thereby greatly reducing the burden of HPV-related diseases in patients.[18,19] However, HPV vaccine-targeted types are limited, and HPV genotypes vary among different regions and populations.[20]

At present, HPV infection and epidemiological characteristics of CA patients in China are rare and incomplete. Therefore,

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in this survey, the evaluation of the epidemic characteristics and HPV genotype distribution of CA cases in Shandong Province, China, will contribute to the adjustment of CA prevention and control strategies, the research and development of HPV vaccine and the formulation of immunization plan.

Materials and methods

Study population

Participants were recruited from patients diagnosed according to the Guideline for the Clinical Management of Anogenital Warts in China (2014),[21] from Shandong Provincial Hospital for Skin Diseases, Shandong Oriental Andrology Hospital, and Jining No.1 People's Hospital from August 2019 to June 2021. None of the recruited patients had ever been vaccinated against HPV. The selection of sentinel hospitals was based on the number of patients diagnosed with CA in the past.

After filling in a questionnaire administered by trained interviewers, each patient underwent sample collection for HPV typing. All patients volunteered to participate in this study and signed the informed consent (Minors under the age of 18 had the consent of their guardians). Our study was approved by the ethics committee of the School of Public Health, Shandong University, China (protocol n. 20190320).

Specimen collection

All patients undergo a clinical examination by a trained clinician at each visit to determine whether there are CA lesions in the cervical, vaginal, vulva, anal, and perianal areas in women or in the external genital, anal, and perianal sites in men. If the CA lesion is found, the sample will be collected from the site by a clinician. Exfoliated cell specimen was collected from the surface of each CA lesion using a sampling brush. The brush was rotated 3–5 full turns in clockwise direction to ensure acquisition of the exfoliated cells. For each patient, each specimen was independently eluted from the sampling brush with normal saline and stored in the refrigerator at −80°C before detecting HPV genotypes.

HPV genotyping

All HPV DNA extraction, amplification and genotype identification were performed with the HPV genotyping panel (polymerase chain reaction (PCR)-reverse dot blot hybridization method; Yaneng Biosciences, Shenzhen, China). The HPV genotyping panel can detect 17 HR-HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, and 82) and 6 LR-HPV genotypes (6, 11, 42, 43, 81, and 83). Genomic DNA was extracted from specimens following manufacturer's protocols. The PCR cycling conditions were as follows: initial denaturation at 95°C for 5 min, then 40 cycles at 94°C for 30 s, 42°C for 90 s, 72°C for 30 s, and a final extension at 72°C for 5 min. HPV type-specific probes are immobilized on nylon membranes for reverse-blot hybridization on, which can detect 23 types of HPV genotypes in a single test. Hybridization was performed and visualized by assessing the protocol according to the manufacturer's instructions. The quality and integrity of the extracted DNA is monitored by the β-actin gene assay. The specimens with known HPV genotypes were used as positive controls and enzyme-free water was used as a negative control.

Statistical analysis

Qualitative categorical variables were described as numbers and percentages. Quantitative variables were described using mean and standard deviation or median and interquartile range (P25-P75), depending on the distribution of the variables. *T*-test was used to compare quantitative variables with a normal distribution, and the Mann-Whitney *U*-test was used to compare quantitative variables without a normal distribution. Chi-square test or Fisher' exact test was conducted to test the hypothesis, as appropriate. All hypothesis tests were two-sided, and *P*-value less than or equal to 0.05 was considered statistically significant. All data were analyzed using SPSS software (version 20.0; IBM Corp.).

Result

CA patients' characteristics

A total of 1185 patients (870 males and 315 females) were included in this study. The Shandong Provincial Hospital for Skin Diseases contributed 52.8% of the total participants, whilst 34.4% and 12.8% were from Jining No.1 People's Hospital and Shandong Oriental Andrology Hospital, respectively. The ages ranged from 15 to 88, with a mean of 34.85 years (SD = 13.60 years). The mean age varied slightly between males and females (35.90 vs. 31.94 years; $p < .01$). Among recruited patients, 63.7% cases reported having more than one sexual partner. The vast majority of patients who reported their sexual preferences were heterosexual (89.1%), including 745 (70.4%) heterosexual men (men who have sex with women, MSW) and 312 (29.5%) heterosexual women. There were 101 patients who were homosexual, including 99 males (men who had sex with men, MSM) and 2 females. In addition, 26 males and 1 female were bisexual who had sex with both males and females. Compared with female patients, male patients had more sexual partners ($p < .001$). In this study, 85.7% of the patients (86.8% of males and 85.0% of females) themselves claimed that their sexual partners were not infected with HPV (Table 1).

Of the 1185 patients with genital warts, one or more subsequent genital warts had been detected in 411 patients (33.1% of males and 39.0% of females). (Table 1) In addition, 32 patients reported other STDs (fifteen with syphilis, five with genital herpes, six with HIV/AIDS, four with gonorrhea and two with chlamydia infection).

The majority of patients presented wart lesions at one location (53.5%), 34.3% patients presented warts at two different locations, 8.8% at three, and 3.4% at four or

Table 1. Baseline characteristics of condyloma acuminatum patients, by gender.

Characteristics	Male (n = 870) n (%)	Female (n = 315) n (%)
Age		
<18	18 (2.1)	13 (4.1)
18-24	202 (23.2)	99 (31.4)
25-29	120 (13.8)	38 (12.1)
30-39	228 (26.2)	92 (29.2)
40-49	146 (16.8)	38 (12.1)
50-59	93 (10.7)	28 (8.9)
≥60	63 (7.2)	7 (2.2)
Marital status		
Single	319 (36.7)	124 (39.3)
Married	532 (61.1)	182 (57.8)
Widowed/Separated/Divorced	19 (2.2)	9 (2.9)
Education		
Primary/Middle school	225 (25.9)	94 (29.8)
High school	209 (24.0)	49 (15.6)
University/College	419 (48.2)	167 (53.0)
Postgraduate	17 (1.9)	5 (1.6)
Area of residence at diagnosis		
Rural area	263 (30.2)	96 (30.5)
City	607 (69.8)	219 (69.5)
Age of first sexual intercourse		
<18	76 (8.7)	23 (7.3)
18-21	431 (49.6)	155 (49.2)
≥22	363 (41.7)	137 (43.5)
Type of CA diagnosis		
Recurrent disease	288 (33.1)	123 (39.0)
New diagnosis	582 (66.9)	192 (61.0)
Condom use		
No	108 (12.4)	34 (10.8)
Yes, sometimes/rarely	676 (77.7)	260 (82.5)
Yes, always	86 (9.9)	21 (6.7)
Weekly frequency of sex		
≤1	397 (45.6)	123 (39.0)
= 2	314 (36.1)	118 (37.5)
≥3	159 (18.3)	74 (23.5)
Wash genitalia before sex		
Yes	543 (62.4)	191 (60.6)
No	327 (37.6)	124 (39.4)
Number of sexual partners		
= 1	288 (33.1)	142 (45.1)
≥2	582 (66.9)	173 (54.9)

more different locations. In females, the CA lesions were often located on the urethral orifice/vaginal introitus (62.9% of the cases), labia majora/labia minora (35.9%), and perianal region (26.4%), whereas, for males, the corona sulcus (36.0%), foreskin (26.4%), and perianal region (23.6%) were the most frequent sites.

HPV prevalence

A total of 880 samples were collected from 659 males and 221 females with a clinical diagnosis of CA. Shandong Dermatology Hospital collected 407 samples, while Jining No.1 People's Hospital and Shandong Oriental Anorectal Hospital collected 321 and 152 samples, respectively. The HPV DNA was detected in 804 of the 880 CA cases, with a positive rate of 91.4% (90.6% males and 93.7% females). Seventy-six samples (from 62 males and 14 females) were excluded from further analysis due to PCR inhibition or insufficient DNA.

Among the 804 patients, 734 (91.3%) patients had at least one LR-HPV infection detected, and 430 (53.5%) patients had HR-HPV infection. In addition, there were 374 (46.5%) patients only infected with LR-HPV, 70 (8.7%) patients were only infected with HR-HPV, and 360 (44.8%) patients were infected with both LR-HPV and HR-HPV. Compared with male patients, female patients had a higher rate of HR-HPV infection ($p < .001$). Moreover, co-infection of LR-HPV and HR-HPV was more common in female patients ($p < .001$). In addition, the prevalence of single HPV genotype infections was 44.2%, while those of multiple HPV genotype coinfections were 55.8%. The total number of HPV genotypes in patients varied from 1 to 12. The frequency of single and multiple infections varied significantly by gender, with single infections being more common among males (47.9% males vs. 33.3% females, $p < .001$) and multiple infections more common among females (52.1% males vs. 66.7% females, $p < .001$). (Table 2)

Distribution of specific HPV genotypes

Overall, all 23 different HPV genotypes were identified in males and females, with a proportion ranging from 1.5% to 57.8% (12/804 to 465/804). The most frequently encountered HPV genotypes were HPV6 (57.8%), HPV11 (37.2%), HPV16 (13.7%), HPV42 (10.3%), and HPV52 (8.8%) by decreasing order of frequency (Figure 1).

Table 2. Distribution of infection types in condyloma acuminatum patients, by gender.

	Males (n = 597)[a] n (%)	Females (n = 207)[a] n (%)	*P* value
HR-HPV	293(49.1)	137(66.2)	<.001
LR-HPV	543(91.0)	191(92.3)	.563
Only HR-HPV	54 (9.0)	16 (7.7)	.563
Only LR-HPV	304 (50.9)	70 (33.8)	<.001
LR and HR HPV	239 (40.0)	121 (58.5)	<.001
Single infection	286 (47.9)	69 (33.3)	<.001
Multiple infections	311 (52.1)	138 (66.7)	<.001
HPV6 or 11	515 (86.3)	188 (90.8)	.088
HPV16 or 18	109 (18.3)	49 (23.7)	.091
Types of 4-valent vaccines[b]	552 (92.5)	196 (94.7)	.279
Types of 9-valent vaccines[c]	566 (94.8)	200 (96.6)	.290

[a]Patients with unqualified HPV results were excluded from the analysis.
[b]The types of 4-valent vaccines conclude HPV 6, 11, 16, or 18.
[c]The types of 9-valent vaccines conclude HPV 6, 11, 16, 18, 31, 33, 45, 52, or 58.

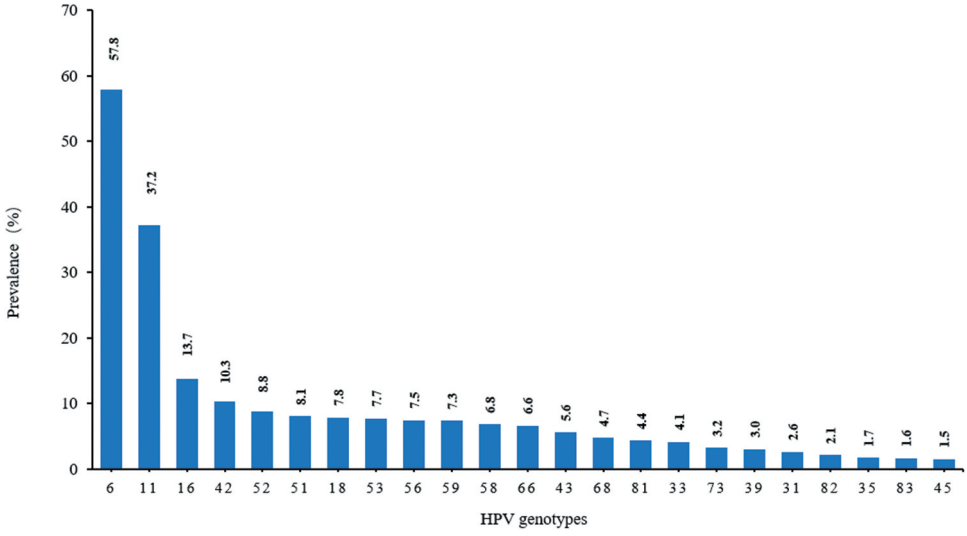

Figure 1. Prevalence of specific human papillomavirus (HPV) infection by decreasing order of frequency. *Patients with unqualified HPV results were excluded from the analysis.

As it was shown, HPV6 was the most common HPV genotype detected in both males (58.1%) and females (57.0%). HPV6/11 (alone or in coinfection with other genotypes) was detected in 87.4% patients, and there was no significant difference between males and females. In addition, there was no significant difference in the distribution of all detective low-risk genotypes between males and females. The most common HR-HPV genotypes were HPV16 (13.7%), HPV52 (8.8%) and HPV51 (8.1%). HPV16/18 (alone or in coinfection with other genotypes) were observed in 19.7% patients. In the HR-HPV genotypes, the frequency of HPV45, HPV56, and HPV58 were significantly higher in women than in men ($p < .05$). Among the patients coinfected with LR-HPV and HR-HPV, the most infection mode is HPV6 + HPV16 (5.6%). Different frequencies of other HPV genotypes in different genders are shown in Table 3.

Discussion

As we all know, CA is one of the most common sexually transmitted diseases (STDs) caused by HPV infection, and most sexually active persons are usually exposed to HPV during their lifetime.[22] In this study based on surveys in sentinel hospitals, we reported the prevalent characteristics of CA and distribution of HPV genotypes in patients with CA in Shandong Province, China. In our study, a total of 1185 CA patients were recruited. The recruited patients were mainly males. This may be due to differences in the anatomical structure of males and females. Compared with males, females need to undergo a professional gynecological examination in the hospital to find out whether they have CA. The average age of CA patients recruited in this study was 34.85 years. Compared with men, women were younger. These results are consistent with a previous Chinese multi-center study of genital warts conducted in 2013 including 1,005 cases from 18 hospitals in seven geographical regions of China.[23] In this study, most patients were in the sexually active stage aged 20 to 40. These patients had risky sexual behaviors such as multiple sexual partners and irregular condom use. Among them, 32 cases also suffered from other sexually transmitted diseases. These are all risk factors that increase HPV infection.[24,25] In addition, 34.7% of the patients in this study had recurrences.

Among the 880 patients who underwent HPV testing, the overall HPV test positivity rate was 91.4%, which shows that HPV is the pathogen that causes CA. There were 8.6% of the patients that could not be included in the analysis due to

Table 3. Prevalence of specific human papillomavirus (HPV) genotypes in condyloma acuminatum patients, by decreasing order of frequency.

HPV genotypes	Males (n = 597)[a] n (%)	Females (n = 207)[a] n (%)	*P* value
LR genotypes			
HPV6	347 (58.1)	118 (57.0)	.779
HPV11	214 (35.8)	85 (41.4)	.181
HPV42	64 (10.7)	19 (9.2)	.530
HPV43	37 (6.2)	8 (3.9)	.208
HPV81	23 (3.9)	12 (5.8)	.237
HPV83	10 (1.7)	3 (1.4)	.824
HR genotypes			
HPV16	77 (12.9)	33 (15.9)	.272
HPV52	51 (8.5)	20 (9.7)	.625
HPV51	43 (7.2)	22 (10.6)	.119
HPV18	43 (7.2)	20 (9.7)	.257
HPV53	44 (7.4)	18 (8.7)	.538
HPV56	36 (6.0)	24 (11.6)	.009
HPV59	44 (7.4)	15 (7.2)	.953
HPV58	34 (5.7)	21 (10.1)	.029
HPV66	36 (6.0)	17 (8.2)	.276
HPV68	24 (4.0)	14 (6.8)	.109
HPV33	20 (3.4)	13 (6.3)	.067
HPV73	23 (3.9)	3 (1.4)	.092
HPV39	17 (2.8)	7 (3.4)	.697
HPV31	16 (2.7)	5 (2.4)	.837
HPV82	12 (2.0)	5 (2.4)	.727
HPV35	8 (1.3)	6 (2.9)	.140
HPV45	5 (0.8)	7 (3.4)	.009

[a]Patients with unqualified HPV results were excluded from the analysis.

insufficient cellular DNA content. Previous studies have found that 16 ~ 26% of the samples will be negative regardless of the type of cell brush sampling.[26,27] Compared with other studies, the lower negative rate of our study reflects the higher quality of sample collection after unified training. In the analysis of specific HPV types, it was shown that the most common genotypes in Shandong Province, China, were HPV6, HPV11, HPV16, and HPV42. Compared with other regions in China, HPV42 had a higher prevalence in Shandong province and had become one of the most common genotypes of HPV in CA patients. Previous studies have found that the distribution of HPV genotypes varies from region to region. Although the risk of diseases related to nonvaccine HPV genotypes is theoretically still low, the actual situation still needs to be monitored to deal with the disease burden caused by high prevalence of nonvaccine HPV genotypes. Furthermore, the distribution of HPV6, HPV11, and HPV16 were consistent with the results of studies in other regions of China.[23,28,29]

In this study, males were mainly infected with LR-HPV, while females were mainly multiple infected with high-risk and LR-HPV. Most patients, both male and female, were infected with more than one different HPV genotype. According to our results, HPV6 and 11 (alone or coinfection with other genotypes) were detected in 87.4% of the patients, but most of these patients also had HR-HPV infection. In this study, 53.5% of the patients with CA were infected with HR-HPV. Among male patients, 49.1% were infected with at least one HR-type of HPV. Some studies have shown that male penile cancer and anal cancer are associated with high-risk HPV infection.[30] Compared with male patients, female patients had a higher infection rate of HR-HPV (66.2% vs. 49.1%), indicating that women had a higher burden of HR-HPV infection. Persistent HR-HPV infection can lead to precancerous lesions and cancers of the cervix, vulva, vagina, and anus in women, so it is very important to prevent the occurrence of these lesions.[31] Some studies have found that high HPV vaccination coverage significantly reduced the prevalence of CA, HPV infection, and cervical intraepithelial neoplasia grade 2+ (CIN2+).[32,33] In addition, when formulating policies to prevent HPV infection in women, it should be considered that men also have a higher infection rate of HR-HPV. Some studies have found that male HPV multiple infection is very common, and the HR-HPV infection rate is the same as that of women.[34–36] There is a higher risk of cross-infection between men and women.[37,38] HPV vaccination in men can effectively reduce related HPV infection rates and HPV-related cancer incidence.[39,40]

Nowadays, four prophylactic HPV vaccines are available in China, including the HPV bivalent vaccine (Cervarix®; Cecolin®) to protect against HPV16 and 18; the HPV quadrivalent vaccine (Gardasil®) to protect against HPV6, 11, 16, and 18; the HPV nonavalent vaccine (Gardasil®9) to protect against HPV6, 11, 16, 18, 31, 33, 45, 52, and 58.[41,42] At present, there are no HPV vaccination programs implemented in China, which may be related to the insufficient data of HPV epidemiological survey. However, the distribution of HPV genotypes varies from region to region.[43] Although vaccines can provide protection for people, it is important to study the prevalence and distribution of HPV genotypes in different regions before implementing the HPV vaccination plan, because understanding the distribution of HPV genotypes will help to choose the best vaccine for HPV protection in each region.[44] In this study, HPV6 and HPV11 were the causes of CA in the majority of patients (87.4%), and they were included in both the quadrivalent and nonavalent vaccines. Considering the economic benefits and the insufficient supply of 9-valent HPV vaccine in China, the 4-valent HPV vaccine may be more suitable for the prevention of CA in Shandong

Province, China. Furthermore,HPV42, as one of the genotypes with higher prevalence in this study, has not been included in any licensed HPV vaccines. Previous studies have found that among people vaccinated with the quadrivalent HPV vaccine, the infection rate of nonvaccine HPV genotypes remains flat or higher.[45] Therefore, it is necessary to develop a broader spectrum of HPV vaccines to reduce the disease burden caused by HPV infection.[46]

In conclusion, CA cases are mainly infected with LR-HPV, but there are more HR-HPV infections in both males and females, which is a problem that cannot be ignored in clinical treatment. It is necessary to monitor the HPV genotype of CA patients, because HR-HPV infection is the main risk factor for cancer. For the general public, HPV typing and HPV vaccination will help to prevent CA or HPV-related cancer and reduce the risk of disease. Additionally, nonvaccine HPV genotypes also need to be monitored for their prevalence by regular HPV typing. The results of our study make a contribution to the epidemiology of HPV in Chinese CA patients by improving the understanding of existing HPV genotypes. Furthermore, this will have a positive impact on the adjustment of CA prevention and treatment strategies, the research and development of HPV vaccines and the formulation of immunization programs.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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Ethics and dissemination

Our study was approved by the ethics committee of the School of Public Health, Shandong University, China (protocol n. 20190320). All patients volunteered to participate in this study and signed the informed consent (Minors under the age of 18 had the consent of their guardians).

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