## ORIGINAL RESEARCH

# Clinical Characteristics of Human Papillomavirus Infection in the Male Genital Tract

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#### ABSTRACT

**Objective** • This study aims to understand the clinical characteristics of male HPV infection and provide data and information for the prevention and health of the male and female reproductive tracts in the region.

**Methods** • A total of 390 male patients who underwent HPV examinations in outpatient clinics and physical examinations in 363 hospitals from December 2017 to May 2022 were selected. Samples were collected, and HPV genotyping was performed using multiplex fluorescent PCR. The HPV infection rate, genotype distribution, age distribution, and clinical symptom distribution were analyzed.

**Results** • Out of 3,816 samples, the total HPV infection rate was 47.44% (185/390). The HPV infection rate in the symptomatic group was 57.09% (141/247), significantly higher than that in the asymptomatic group (P < .01). Among the subtypes, HPV6 accounted for the highest proportion (31.03%, 90/290), followed by HPV11 (14.14%, 41/290) and HPV52 (8.62%, 25/290). Types 6 and 11 were

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#### INTRODUCTION

Human papillomavirus (HPV) is a spherical DNA virus that exhibits a specific affinity for epithelial cells. It is one of the most prevalent sexually transmitted infections worldwide. Currently, more than 200 subtypes of HPV have been identified, with approximately 40 subtypes associated with genital infections. HPV infections are primarily transmitted through sexual contact and affect both males and females.<sup>1</sup> HPV is primarily transmitted through various forms of sexual contact, including vaginal, anal, and oral sex. In females, HPV infection is strongly associated with the development of cervical cancer, which poses a significant mainly concentrated in the symptomatic group (91.11%, 85.37%). The highest positive rate was observed in the 17-30-year-old group (45.41%, 85/185), followed by the 31-40-year-old group (28.11%, 52/185). The proportion of HPV infections with clinical symptoms of abnormal growth was 84.40% (119/141). HPV6 or/and HPV11 infections were mainly concentrated in the abnormal growth group, accounting for 90.76% (108/113).

**Conclusions** • The rates of male HPV infection are high, particularly among individuals aged 17-40. Low-risk infections (types 6 and 11) cause male reproductive tract symptoms, including abnormal growth. High-risk infection (HPV52) correlates with local women's HPV subtype distribution and potential transmission. Therefore, screening for male HPV infection is crucial in preventing cervical cancer. Authorities should promote the development and early use of male HPV vaccines. (*Altern Ther Health Med.* 2023;29(6):425-429).

public health concern. Globally, cervical cancer ranks as the fourth most common cancer among women. Almost all cases of cervical cancer are estimated to be caused by HPV infections, particularly those involving high-risk HPV subtypes.<sup>2</sup> Apart from cervical cancer, HPV infection can also lead to other types of cancer, including anal, vaginal, vulvar, penile, and oropharyngeal cancers. The majority of these cancer cases are attributed to high-risk HPV subtypes.<sup>1-3</sup> The National Medical Products Administration has approved the use of the 9-valent HPV vaccine for women aged 9-45 years,<sup>4</sup>, which holds significant implications for preventing cervical cancer in women.

Despite the well-established association between HPV and cervical cancer, there remains a relative lack of research on the clinical characteristics of HPV infection in males, particularly in China. Understanding the epidemiology and severity of HPV-related diseases in males is crucial for implementing effective prevention and treatment strategies. Our cross-sectional study aims to provide insights into HPV infection in males, including subtype distribution and clinical symptoms, to contribute to a better understanding of this important public health issue.

## MATERIALS AND METHODS

#### Study Design

The study design employed in this research is a crosssectional study, allowing for the analysis of HPV infection rates, genotype distribution, age distribution, and clinical symptom distribution in a specific population of male patients. This design enables the examination of current characteristics and associations, providing valuable insights into the epidemiology and clinical manifestations of HPV infection in males.

#### **Study Subjects**

A total of 390 males who underwent HPV testing at the outpatient clinic and physical examination in Sanliu Hospital between December 2017 and May 2022 were enrolled in this study. The age of the subjects ranged from 13 to 86 years. Based on the presence or absence of genital symptoms, the study population was divided into two groups: a symptomatic group (247 cases) and an asymptomatic group (143 cases), according to the medical records. Informed consent was taken from all patients who willingly agreed to undergo HPV genotyping testing.

#### **HPV Detection Methods and Equipment**

For HPV detection, a fluorescent PCR method utilizing the HPV (23 types) detection kit from Kaipu in Chaozhou was employed. The PCR amplification was conducted using an Applied Biosystems 7500 PCR amplification instrument. Nucleic acid extraction was performed using the HBNP-4801A nucleic acid extractor from Kaipu in Chaozhou.

#### Sample Collection and Processing

Sample collection and processing includes following: (1) urethral secretions: clinical doctors obtained urethral secretions by gently rotating a sterile swab approximately 2-3 cm inside the urethral opening; (2) wound secretions: clinical doctors collected secretions and cells from skin lesions on the foreskin, coronal sulcus, prepuce, and perianal area using a sterile swab; (3) proliferative abnormal tissue: clinical doctors collected abnormal tissue that had proliferated on the foreskin, coronal sulcus, prepuce, and perianal area in a sterile cup. The tissue was then cut into "rice grain" shapes using sterile scissors; (4) sample processing: the collected swabs or tissue samples were washed with saline, and DNA extraction was performed using a nucleic acid extraction instrument.

### **HPV** Genotyping

For HPV genotyping, specific primers and probes were designed to target the L1 region of the HPV genome. Additionally, primers and probes were designed against  $\beta$ -globin as a reference gene. The reaction was divided into six reaction tubes. Multiple fluorescence PCR technology was employed to genotype 23 types of HPV, which included 17 high-risk HPV (hrHPV) subtypes: HPV16, 18, 31, 33, 35, 39,

45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 82, and 6 low-risk HPV (lrHPV) subtypes: HPV6, 11, 42, 43, 44, 81.

The PCR reaction system (50  $\mu$ L) consisted of pH 8.3 Tris-HCl (1 mol/L) 3.25  $\mu$ L, ammonium sulfate (0.5 mol/L) 1.66  $\mu$ L, Tween-20 0.25  $\mu$ L, magnesium acetate bovine serum albumin (5 mmol/L) 10  $\mu$ L, Taq enzyme 5 U, dNTPs (25 mmol/L) 0.6  $\mu$ L, each subtype primer 3 pmol/L, template DNA 5  $\mu$ L, and ddH2O was added to make up 50  $\mu$ L. The PCR reaction conditions were as follows: 95°C for 10 min, 95°C for 15 s, 60°C for 60 s, for 45 cycles, followed by 38°C for 5 min. Hela cells were used as positive controls, while water served as negative controls. A Ct value of less than 40 was considered positive. Each well contained an internal control.

#### **Statistical Analysis**

Statistical analysis was conducted using Statistical Product and Service Solutions (SPSS) version 23.0 (IBM, Armonk, NY, USA). Normally distributed continuous variables were presented as mean  $\pm$  standard deviation. The differences between groups for normally distributed continuous variables were assessed using a *t* test. Rates or composition ratios were compared using either a chi-square test or Fisher's exact test. A significance level of *P* < .01 was considered statistically significant.

#### RESULTS

#### **HPV Infection Status**

Among the 390 participants, the overall HPV infection rate was 47.44% (185/390), with 23.59% (92/390) attributed to hrHPV and 36.15% (141/390) to lrHPV. The difference between the two subtypes was statistically significant ( $\chi^2$  = 14.694, *P* = .000). Within the HPV-infected population, 62.16% (115/185) had single subtype infections, while 37.84% (70/185) had multiple subtypes. There were statistically significant differences in HPV infection rates, hrHPV infection rates, and lrHPV infection rates between the symptomatic and asymptomatic groups (*P* < .01). However, the two groups had no statistically significant differences in the proportion of single or multiple subtype infections (*P* > .01). Please refer to Table 1 for specific results.

 Table 1. HPV infection status in 390 male participants

Study Population	Symptomatic Group	Asymptomatic Group	<b>X</b> <sup>2</sup>	P value
Number of Cases (n)	247	143	-	-
Age (Years)	33 ± 13	$34 \pm 10$	-	-
HPV Infection Rate	57.09%(141/247)	30.77%(44/143)	25.154	.000
Hrhpv Infection Rate	25.91%(64/247)	11.33%(28/143)	17.311	.000
Lrhpv Infection Rate	48.58%(120/247)	15.38%(22/143)	94.918	.000
Rate of Single Infection	60.99%(86/141)	68.18%(30/44)	0.741	.389
Rate of Multiple	39.01%(55/141)	31.82%(14/44)		
Infections				

**Abbreviations**: HPV, Human papillomavirus;  $\chi^2$  (Chisquare), Statistical test used for comparison; hrHPV, Highrisk HPV; lrHPV, Low-risk HPV.

### **Positive Rates of Different Sample Types**

Among the 390 samples, significant differences were observed in the HPV positivity rates among urethral secretions, proliferative lesions, and wound secretions (P < .01). Proliferative lesions exhibited the highest positivity rate (68.00%), followed by urethral secretions (34.05%), while wound secretions had the lowest positivity rate (10.00%). For detailed information, please refer to Table 2.

#### **Distribution of HPV Subtypes**

Among the 185 individuals with HPV infection, a total of 290 HPV subtype frequencies were identified due to 70 cases of multiple subtype infections. All 23 subtypes were present, with HPV6 being the most common (31.03%, 90/290), followed by HPV11 (14.14%, 41/290) and HPV52 (8.62%, 25/290). In the symptomatic group, the total frequency of HPV subtypes was 226, which was significantly higher than that in the asymptomatic group (P <.01). The top three subtypes in the symptomatic group were HPV6 (36.28%, 82/226), HPV11 (15.49%, 35/226), and HPV52 (7.08%, 16/226). No significant differences were observed in the distribution of HPV subtypes among the asymptomatic group. For detailed information, please refer to Table 3.

Regarding male HPV6-infected patients, they were predominantly found in the symptomatic group, accounting for 91.11% (82/90), which was significantly higher than that in the asymptomatic group (P < .01). Male HPV11-infected patients were also mainly concentrated in the symptomatic group, representing 85.37% (35/41), which was significantly higher than that in the asymptomatic group (P < .01). However, there was no significant difference in the distribution of male HPV52-infected patients between the symptomatic and asymptomatic groups (P > .01). Refer to Table 4.

### HPV Infection Distribution by Age Group

The 390 patients were categorized into six age groups: <17 years, 17-30 years, 31-40 years, 41-50 years, 51-60 years, and 60 years and above. The highest HPV-positive rate was observed in the 17-30 years group, followed by the 31-40 years group, with a statistically significant difference between

#### Table 2. HPV positivity rates in different specimen types.

Sample Type Urethral Secretion		Abnormal Proliferation Tissue	Wound Secretion	χ <sup>2</sup>	P value
Number of Cases (n)	185	175	30	-	-
Positive Rate (%)	34.05 (63/185)	68.00 (119/175)	10.00 (3/30)	59.828	.000

**Abbreviations**: HPV, Human papillomavirus;  $\chi^2$  (Chi-square), Statistical test used for comparison.

**Table 3.** Distribution of HPV Subtypes in the Male Population with HPVInfection.

	Number of Positive Cases (n)				Number of Positive Cases (n)		
	Symptomatic	ic Asymptomatic			Symptomatic Asymptomati		
Subtype	Group	Group	Total	Subtype	Group	Group	Total
HPV6	82	8	90	HPV81	2	5	7
HPV11	35	6	41	HPV18	4	2	6
HPV52	16	9	25	HPV43	4	2	6
HPV16	12	5	17	HPV31	5	0	5
HPV51	9	2	11	HPV33	3	2	5
HPV53	8	3	11	HPV82	4	0	4
HPV58	8	3	11	HPV56	3	0	3
HPV42	8	2	10	HPV35	2	0	2
HPV39	5	3	8	HPV73	2	0	2
HPV44	5	3	8	HPV45	1	0	1
HPV59	6	2	8	HPV68	0	1	1
HPV66	2	6	8	Total	226	64	290

#### Abbreviations: HPV, Human papillomavirus.

**Table 4.** Distribution Differences of the Top Three HPV Subtypes betweenSymptomatic and Asymptomatic Groups.

HPV subtypes	Symptomatic Group	Asymptomatic Group	$\chi^2$	P value
HPV6	82(91.11%)	8(0.89%)	121.689	.000
HPV11	35(85.37%)	6(14.63%)	41.024	.000
HPV52	16(64.00%)	9(36.00%)	3.920	.048

**Abbreviations**: HPV, Human papillomavirus;  $\chi^2$  (Chi-square), Statistical test used for comparison.

Table 5. Distribution of HPV Infection in Different Age Groups.

Age Group		Number of	Distribution of Positive	Number of Symptomatic	Distribution of Symptomatic
(Year)	n	Positive	Patients (%)	Patients	Patients (%)
<17	1	1	0.54	1	0.40
17-30	192	84	45.41	133	53.85
31-40	115	52	28.11	63	25.51
41-50	38	20	10.81	19	7.69
51-60	27	17	9.18	16	6.48
Above 61	17	11	5.95	15	6.07
Total	390	185	100.00	247	100.00

Abbreviations: n, Number of Cases; HPV, Human papillomavirus.

Number Of **Positive Patient** Number Of Cases With HPV6 HPV6 and/or HPV11 **Clinical Symptoms Positive Cases** and/or HPV11 Infection n Distribution (%) Infection Rate (%) Abnormal Growth 174 119 84.40 108 90.76 Genitourinary Infection 29 11 7.80 3 27.27 Erythematous Papules 17 2 1.42 0 0.00 Balanoposthitis 14 6 4.26 1 16.67 7 2 Ulceration 1.42 0.50 1 3 0 Tumor 1 0.71 0.00 Herpes Simplex Virus Infection 3 0 0.00 0 0.00 Total 247 141 100.00 113 100.00

 Table 6. Distribution of Clinical Characteristics of HPV Infection.

Abbreviations: n, Number of Cases; HPV, Human papillomavirus.

the two groups ( $\chi^2$  = 12.623, *P* = .000). Male HPV infection was predominantly concentrated in the 17-30 years and 31-40 years age groups, accounting for 73.52% (136/185) of cases. Similarly, male patients with HPV infection symptoms were primarily found in the 17-30 years and 31-40 years age groups, comprising 79.35% (196/247) of cases. Refer to Table 5.

#### **Distribution of HPV Infection in Clinical Symptoms**

Among the 247 symptomatic male patients, they were categorized into seven groups based on their clinical symptoms at the time of diagnosis: abnormal growth group, urinary tract infection group, erythematous papule group, balanoposthitis group, ulceration group, tumor group, herpes group. Male HPV infection was predominantly concentrated in the abnormal growth group, accounting for 84.40% (119/141) cases. HPV6 and/or HPV11 infections were primarily found in the abnormal growth group, representing 90.76% (108/113) cases. Refer to Table 6 for further details.

#### DISCUSSION

## The Significance and Limitations of Research on Male HPV Infection in China

Following HPV infection in the reproductive tract, most cases are cleared by the immune system or present with subclinical symptoms. However, a small number of persistent cases can lead to proliferative damage in the squamous epithelial cells of the genital tract and adjacent skin and mucosa, resulting in conditions such as genital warts, cervical cancer, and penile cancer.<sup>5,6</sup> Research on the relationship between HPV infection and women's reproductive health, particularly cervical cancer, has garnered significant attention worldwide. In contrast, studies on male HPV infection, a crucial source of infection for females<sup>7</sup> and associated with penile cancer, anal cancer, genital warts, and other diseases, have not received sufficient attention in China.

Furthermore, prevention strategies for male HPV infection differ between China and other countries. The US Food and Drug Administration approved the use of the nine-valent HPV vaccine for females and males under the age of 45 in 2018.<sup>8</sup> However, due to a lack of relevant research data, no vaccines are currently available for preventing male HPV infection in China. Therefore, further research on the HPV infection status among male populations is of utmost

significance for treating and preventing reproductive health in both males and females.

### Epidemiological Research Findings and Analysis of HPV Infection in Males

The findings of this study revealed that among the 390 participants, the HPV infection rate was 47.44%, which was higher than the reported rate of 47.60% in a previous study conducted in Changchun, China, involving hot springs.<sup>9</sup> Furthermore, it was higher than the HPV infection rate of 39.18% reported in male patients at Tongde Hospital in Zhejiang Province by Zhang Yongle et al.<sup>10</sup> However, it was lower than the HPV infection rates of 58.33% reported in Dongguan by Zhong Yangqing et al.<sup>11</sup> and 64.3% reported in southeast Beijing by Zhang Xinpeng et al.<sup>12</sup> These variations could be attributed to differences in participant selection.

Our study found a higher HPV infection rate in the symptomatic group (57.09%) compared to the asymptomatic group ( $\chi^2 = 25.154$ , P = .000), which aligns with the findings in Dongguan by Zhong Yangqing et al.<sup>11</sup> Variations could also influence the discrepancies in sampling methods. Our study revealed a significant difference in HPV positivity rates among urethral secretions, foreign body tissue, and wound secretions ( $\chi^2 = 59.828$ , P = .000), with foreign body tissue displaying the highest positivity rate (68.00%), followed by urethral secretions (34.05%) and wound secretions (10.00%). Furthermore, differences in living habits and hygiene conditions across regions may contribute to the observed variations.<sup>10-11</sup>

## Subtype Distribution and Clinical Implications of HPV Infection in Males

The distribution of HPV subtypes in male HPV infection can vary across countries and regions, and different subtypes may lead to distinct clinical symptoms. In our study involving 390 men primarily from Chengdu, Sichuan Province, we detected 185 individuals with HPV infection, resulting in a total of 290 HPV subtypes. The most prevalent subtype was HPV6, followed by HPV11 and HPV52. These findings align with the results reported in Hainan by Lv Jiaqing et al.<sup>13</sup> but differ from the findings in Vietnam by Hoai et al.<sup>14</sup> (types 11, 6, and 16) and Jinan by Xiao Hailu et al.<sup>15</sup> (types 6, 11, and 16). However, these studies have consistently shown that low-risk HPV infections in males are primarily caused by types 6 and 11.

In our study, we found that the positivity rates of types 6 and 11 in the symptomatic group were significantly higher than those in the asymptomatic group ( $\chi^2 = 121.689$ , P = .000;  $\chi^2 = 41.024$ , P = .000), indicating that types 6 and 11 are more likely to cause male reproductive system lesions and clinical symptoms. Hence, early treatment and prevention measures are crucial. High-risk HPV infection was most commonly associated with type 52, and the positivity rate did not significantly differ between the symptomatic and asymptomatic groups. This finding suggests that type 52 may cause subclinical infections in males, although we suspect it is influenced by the fact that type 52 is the most prevalent subtype among women in the local area. Therefore, in addition to focusing on preventing and treating HPV infection in women for cervical cancer prevention, it is equally important to emphasize prevention and treatment in the male population to achieve comprehensive and effective prevention, reduce recurrence, and minimize reinfection.

### Age Distribution, Symptom Characteristics, and Preventive Measures of HPV Infection in Males

Our research findings indicate that the male population with HPV infection in our study was primarily concentrated in the age range of 17-40 years (accounting for 73.52%), which is consistent with existing literature reports.<sup>12</sup> Similarly, patients with symptoms were predominantly found within this age group, representing 79.35% of cases. The HPV infection rate in males aged 17-30 was significantly higher than in the 31-40 age group. This observation can be attributed to the sexual activity and frequency of changing sexual partners among males in this age range. Hence, it is crucial not to overlook this particular age group and to strengthen HPV prevention and treatment efforts. This can be achieved through enhanced sex education, the promotion of HPV knowledge, and the implementation of measures to minimize the occurrence of male reproductive tract diseases and the transmission of HPV to the female reproductive tract.

We conducted a classification and analysis of the symptomatic group based on different clinical symptoms, and our findings revealed that HPV-positive males predominantly belonged to the group with abnormal proliferation, accounting for 84.40% of cases. This group was mainly infected with HPV6 and/or HPV11. Notably, among the cases examined, an infant aged 13 months exhibited multiple abnormal proliferations in the genital tract. Subsequent sampling and HPV subtype testing confirmed a positive result for HPV6, indicating that HPV can cause reproductive tract diseases not only in sexually active adults but also in infants and young children. Furthermore, we identified one case of a penile tumor patient who tested positive for HPV16. These findings emphasize the importance of promoting the early use of HPV vaccines for males, particularly targeting types 6 and 11. Relevant authorities and departments should advocate such measures.

#### **Study Limitations**

Several limitations should be considered when interpreting the results of this study. Firstly, the study was

conducted in a specific geographical region and may not represent the entire population. The sample size was also relatively small, which could limit the generalizability of the findings. Additionally, the study relied on self-reported clinical symptoms, which may introduce recall bias. The cross-sectional design of the study limits our ability to establish causal relationships and draw definitive conclusions. Furthermore, the study focused on male HPV infection and its clinical characteristics, neglecting other potential factors that could influence HPV transmission and disease outcomes. Despite these limitations, the study provides valuable insights into the prevalence and subtype distribution of HPV infection in males, contributing to the existing knowledge in this field.

#### CONCLUSION

The prevalence of HPV infection in males is notably high, particularly within the age group of 17-40 years. Low-risk infections, primarily attributed to HPV types 6 and 11, are strongly associated with male genital symptoms, particularly abnormal growths. On the other hand, high-risk infections are predominantly caused by HPV type 52, which aligns with the subtype distribution observed in local females. These findings suggest a potential correlation between the prevalence of HPV type 52 transmission and high-risk infections in males.

#### CONFLICT OF INTEREST

The authors have no potential conflicts of interest to report relevant to this article.

#### **AUTHORS' CONTRIBUTIONS**

TG, PL and LZ designed the study and performed the experiments; TG, PL, and XY collected the data; LZ and XY analyzed the data; TG, PL and LZ prepared the manuscript. All authors read and approved the final manuscript. TG and PL contributed equally to this work.

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