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# Epidemiological Investigation of Human Papillomavirus Infection in Men Attending a Sexually Transmitted Disease Clinic in Hangzhou Area[1]

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**Objective** To investigate the epidemiological characteristics of human papillomavirus (HPV) infection in men attending a sexually transmitted diseases (STD) clinic in Hangzhou area. **Methods** Male subjects ($n$=375) aged 18-70 years, attending the STD clinic were recruited. Urethral swabs were assessed for HPV DNA using polymerase chain reaction (PCR) with the consensus primers MY09/11. HPV genotypes of positive PCR products were determined by restriction fragment length polymorphisms and direct sequence analysis. **Results** Of the 375 swabs collected, 305 (81.3%) yielded sufficient DNA for the subsequent HPV analysis. Among the 305 subjects, the prevalence of HPV was 13.8%. Nononcogenic HPV types were found in 8.5% (26/305) of subjects, oncogenic types in 4.3% (13/305), and multiple types in 1.0% (3/305). The prevalence of HPV infection was higher in subjects from urban area than in those from rural area ($P$<0.05). The prevalence was also higher in those who received fewer years of education ($P$<0.05) and those who had more sex partners ($P$<0.05). **Conclusions** HPV infection among men at high risk is not uncommon. The detection rate of HPV DNA is significantly related to some sociodemographic factors, such as residence, educational level and the number of sex partners.

**Key words**: Human papillomavirus; Men; Sexually transmitted diseases

## INTRODUCTION

Human papillomavirus (HPV) infection in women has been studied extensively, and sufficient evidence shows that HPV infection is closely associated with cervical intraepithelial neoplasia and cervical cancer. However, data on HPV infection in men, especially in men at high risk of sexually transmitted disease (STD), are very limited. Recent epidemiological studies have revealed that infection of high-risk type HPVs (cancer causing) in men is closely linked with some anogenital malignancies, including Bowen's disease, erythroplasia of Queyrat, penile cancer, and anal cancer[1]. Moreover, HPV infection in men is overwhelmingly subclinical, which results in a potentially large number of asymptomatic carriers. These carriers might serve as "reservoirs" and "vectors" for the virus, thus increasing the risk of their female sex partners for HPV infection, even cervical cancer[2]. The purpose of our investigation was to assess the prevalence and type distribution of HPV, as well as some other epidemiological characteristics, in men attending a STD clinic in Hangzhou area, eastern China.

## STUDY SUBJECTS AND METHODS

### *Study Subjects*

A total of 375 male subjects between the ages of 18 and 70 years attending a STD clinic in the 3rd Hangzhou People's Hospital between September 2003 and April 2004 were recruited in this study. An informed consent document approved by Human Subject Committee of our hospital was reviewed and signed by each subject. All the subjects answered questionnaire that assessed sexual history, risk factors for STDs, and demographic information. Clinical examinations were performed by physicians experienced in the field of STDs. Each subject first underwent laboratory analysis for gonorrhea, syphilis,

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Chlamydia, and mycoplasma infection.

*Collection of Specimens and Extraction of DNA*

For each subject, a urethral swab was inserted 2 cm into the urethral meatus and rotated 360°. After being inserted into a centrifugal tube containing 2 mL of normal saline solution, each swab was squeezed and rinsed for 1 min to make DNA dissolve sufficiently in the solvent. The suspension was centrifuged at 3000 rpm for 5 min and the supernatant was discarded. DNA was extracted from the cell precipitate using UNIQ-10 centrifugal column viral DNA extraction kit (Shanghai Sangon, China).

*HPV DNA Amplification*

In order to identify viral DNA, PCR was performed using the HPV L1 consensus primers MY09 and MY11. MY11: 5′-GCM CAG GGW CAT AAY AAT GG-3′, MY09: 5′-CGT CCM ARR GGA WAC TGA TC-3′. The quality of total human DNA template was examined by using primers for human β-globin gene, PC03 and PC04. PC03: 5′-ACA CAA CTG TGT TCA CTA GC-3′; PC04: 5′-CAA CTT CAT CCA CGT TCA CC-3′ (110 bp). Standard precautions were taken to prevent contamination during sampling and subsequent processing. Positive HPV controls (plasmid pTHPV16-L1 containing HPV 16 L1 sequence, from National Institute for Viral Disease Control and Prevention, China) and a negative control ($H_2O$) were also amplified using the same procedure (with all experimental and control PCR amplifications repeated at least twice). The amplifications were conducted in a volume of 50 μL, in a reactive mixture containing 0.8 μg DNA, 200 μmmol/L dNTP, 1μmmol/L primers, 2U Taq polymerase, and 5 μL 10×PCR buffer. The samples were subjected to preliminary denaturation for 5 min at 94℃, followed by 30 cycles at 94℃ for 30 s, at 55℃ for 40 s, and at 72℃ for 90 s. The PCR products were analyzed by electrophoresis in 1.5% agarose gel and visualized under ultraviolet light.

*HPV Genotyping and Sequence Analysis*

HPV genotypes of the MY09/MY11 PCR products were determined by restriction fragment length polymorphisms (RFLP) with three restriction enzymes, Rsa I, Pst I, and HaeIII, separately. The digested PCR fragments were separated on 2% agarose gel. For those PCR products whose genotype could not be determined by RFLP, the purified PCR products were ligated into the vector pGEMT-T (Promega) and verified by sequence analyses. The obtained sequence was compared with the published HPV sequences in Genbank.

*Data Analysis*

Statistical analysis was performed by means of the Chi-square test.

## RESULTS

Approximately 75.0% of 500 patients agreed to be recruited in the study, resulting in the enrollment of 375 participants. The majority of the subjects complained about genitourinary symptoms, e. g. abnormal penile secretion or a feeling of discomfort (45.6%) and verruca or blister in genitals (15.2%). Some others participated because they "just wanted to get checked" (25.9%), or they "were once exposed to sex partners with STD" (7.5%). Of the 375 swabs collected, 305 (81.3%) yielded sufficient DNA for the subsequent HPV analysis. Among the 305 subjects, the prevalence of HPV DNA was 13.8%. Selected demographic and sexual behavior characteristics of the subjects, along with prevalence of HPV infection, are shown in Table 1. The mean age of subjects was 28.5±8.3 years, with a range of 18-70 years. Statistically significant associations with HPV infection were observed on residence, educational level, and the number of sex partners. The HPV prevalence was higher among the subjects from urban area, fewer years of education, or having more sex partners.

Table 2 presents the prevalence of genital HPV in urethral swabs, with the classification of HPV into oncogenic and nononcogenic types. Nononcogenic HPV types were found in 8.5% (26/305) of subjects, oncogenic types in 4.3% (13/305), and multiple types in 1.0% (3/305). Overall, the most prevalent types of HPV were nononcogenic type 6 (6.2%) and type 11 (1.6%). The most common monogenic types of HPV wene type 16 (2.3%) and type 18 (1.0%). In multiple infections, two cases showed HPV6+11, and one case HPV6+16.

Table 3 shows the association between prevalent HPV infection and other concurrent STDs. Nongonococcal urethritis was the most commonly diagnosed STD, with a prevalence of 19.0%. The only significant association was found between HPV infection and the current presence of genital warts (continuity correction $\chi^2$ =12.629, $P$=0.000). HPV DNA was detected in 11 cases of 30 genital warts, of whom 8 with HPV 6 and 3 with HPV 11.

TABLE 1

HPV DNA Prevalence in Men in Hangzhou Area: Association With Demographic and Sexual Behavior Characteristics

| Characteristics | No. | Percent (%) | No. HPV DNA Positive | Positive Rate |
|---|---|---|---|---|
| Age (Years) | | | | |
| 18 | 6 | 2.0 | 1 | 16.7 |
| 19-29 | 118 | 38.7 | 19 | 16.1 |
| 30-40 | 134 | 43.9 | 17 | 12.7 |
| 40-70 | 47 | 15.4 | 5 | 10.6 |
| Residence[a] | | | | |
| Urban | 242 | 79.3 | 39 | 16.1 |
| Rural | 63 | 20.7 | 3 | 4.8 |
| Marital Status | | | | |
| Married | 131 | 42.9 | 25 | 19.1 |
| Single | 92 | 30.2 | 11 | 12.0 |
| Cohabitating | 35 | 11.5 | 4 | 11.4 |
| Divorced/Widowed | 47 | 15.4 | 2 | 4.3 |
| Educational Level[b] | | | | |
| College or Above | 96 | 31.5 | 7 | 7.3 |
| High School | 120 | 39.3 | 16 | 13.3 |
| High School Not Completed | 89 | 29.2 | 19 | 21.3 |
| Sexual orientation | | | | |
| Heterosexual | 295 | 96.7 | 41 | 13.9 |
| Homosexual/Bisexual | 10 | 3.3 | 1 | 10.0 |
| Age at 1st Sexual Intercourse (Years) | | | | |
| ⩽18 | 43 | 14.1 | 8 | 18.6 |
| 19-29 | 237 | 77.7 | 29 | 12.2 |
| ⩾30 | 25 | 8.2 | 5 | 20 |
| No. of Sex Partners[c] | | | | |
| 1 | 151 | 49.5 | 13 | 8.6 |
| 2-5 | 142 | 46.6 | 26 | 18.3 |
| ＞5 | 12 | 3.9 | 3 | 25 |
| Years of Sexual Behavior | | | | |
| ＜10 | 173 | 56.7 | 24 | 13.9 |
| ⩾10 | 132 | 43.3 | 18 | 13.6 |

*Note.* [a]Pearson Chi-square test. $\chi^2 = 5.427$, $P$=0.020. [b]Pearson Chi-square test. $\chi^2 = 7.717$, $P$=0.021. [c]Pearson Chi-square test, $\chi^2 = 7.126$, $P$=0.028.

## DISCUSSION

Contrary to women, it is difficult to determine genital HPV infection in men because the lesions are not always typical and mostly asymptomatic. In addition, there are no such examination and detection methods available in men as in women, such as colposcopy and cervical scraping smear. Because of the diversity of life style, hygienic situation and detection techniques, various infection rates have been reported in different nations or areas. The USA STD clinics reported that the HPV prevalence among men is 28.2%[3], whereas a Swedish STD clinic reported an HPV prevalence of 13%[4]. It was reported that the HPV prevalence in Denmark is 45% - 49%[5]. In an international case-control study on the HPV prevalence among the male partners of women with and without cervical cancer, the International Agency for Research on Cancer (IARC) investigators found that the penile HPV DNA is 3.5%-39% in the control husbands and 12.0%-36.0% in the husbands of the patients, with a prevalence varying significantly by countries[6-7].

TABLE 2

Prevalence of HPV Types

| HPV Type | No. Positive | Prevalence Rate |
|---|---|---|
| Nononcogenic (Total) | 26 | 8.5 |
| 6 | 19 | 6.2 |
| 11 | 5 | 1.6 |
| 42 | 1 | 0.3 |
| 54 | 1 | 0.3 |
| Oncogenic (Total) | 13 | 4.3 |
| 16 | 7 | 2.3 |
| 18 | 3 | 1.0 |
| 31 | 1 | 0.3 |
| 33 | 2 | 0.7 |
| Multiple | 3 | 1.0 |

TABLE 3

Male HPV Infection Concurrent With Other STDs

| Other STDs | No. of Subjects | Percent (%) | No. of HPV-DNA Positive | Positive Rate |
|---|---|---|---|---|
| Gonorrhea | | | | |
| Positive | 31 | 10.2 | 5 | 16.1 |
| Negative | 274 | 89.8 | 37 | 13.5 |
| Nongonococcal Urethritis | | | | |
| Positive | 58 | 19.0 | 11 | 19.0 |
| Negative | 247 | 81.0 | 31 | 12.6 |
| Syphilis | | | | |
| Positive | 24 | 7.9 | 2 | 8.3 |
| Negative | 281 | 92.1 | 40 | 14.2 |
| Condyloma Acuminatum[a] | | | | |
| Yes | 30 | 9.8 | 11 | 36.7 |
| No | 275 | 90.2 | 31 | 11.3 |
| Genital Herpes | | | | |
| Yes | 11 | 3.6 | 1 | 9.1 |
| No | 294 | 96.4 | 41 | 13.9 |

*Note.* [a]continuity correction. $\chi^2 = 12.629$, $P = 0.000$.

In our study, specimen adequacy was determined by amplifying a target sequence from the human β-globin gene. Of the 375 urethral swabs collected, 305 (81.3%) yielded sufficient DNA for the subsequent HPV analysis, proving the efficiency of the swab method in collecting urethral epithelial cells. Among the swabs that yielded adequate DNA, 13.8% were HPV DNA positive, which revealed the epidemiological state of HPV infection in men to some extent. Some researchers have used noninvasive sampling of genital skin or urine to identify individuals with subclinical HPV infection. Although the method is non-traumatic, simple and well tolerated, it is not so sensitive to make it a useful means for screening[8-9].

We found that penile HPV DNA prevalences were significantly related to the subject's residency, educational level, and number of sex partners. Namely, those men who had more sex partners, lived in urban area and had received less education had a higher detection rate of HPV DNA; whereas the other sociodemographic and sexual behavior characteristics were not found to have any statistical relationship with the detection rate of HPV DNA, including age, marital status or sexual orientation, *etc*. Other investigators also reported that age is not associated with HPV prevalence among men[7-8,10], though in women the prevalence of HPV declines with

increasing age, with a smaller postmenopausal peak[11-12]. This disparity between men and women might reflect a sex-based variation in immune responses to the virus.

Condyloma acuminatum is the only STD associated with penile HPV infection. In our research, the HPV DNA detection rate of urethral swab samples from the subjects with condyloma acuminatum was 36.7% (11/30), significantly higher than that from those without condyloma acuminatum, suggesting that the male urethra may serve as a reservoir for HPV and contribute to the recurrence of condyloma acuminatum after therapy.

In women, the oncogenic HPV types, especially type 16, are more commonly detected than the nononcogenic types[13-14]. In our research, the nononcogenic HPV types occurred more frequently in men than the oncogenic types. In the past decade, large amounts of epidemiological evidence have confirmed that men, particularly those "high risk males" with oncogenic HPV infection in the urethra, play an important role in the development of cervical cancers in women. Castellsague *et al*.[2] hold that men who have multiple sex partners may be vectors of high-risk HPV types to transfer the virus from sex workers to his wife or other sex partners and consequently increase their risk of cervical cancers.

In conclusion, HPV infection in men attending a STD clinic is not rare in Hangzhou area. Although the symptoms of HPV infection in men may not be so noticeable as in women, high risk men do act as carriers and "vectors" of HPVs to increase their sex partners' risk of condyloma acuminatum, even cervical cancer. In addition, some studies indicate that oncogenic HPVs contribute to the development of anogenital cancers in men. Therefore, it is necessary to prevent and control this disease in men. Moreover, identification of the dominant types of HPV in men unquestionably facilitate the establishment and application of the vaccine of HPV in the near future.

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