

Original Article

HPV in men and concordance of viral types in infected couples in southern Mexico

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Abstract: The HPV infection in men is still important because the associated risk factors with infection are controversial and by its role in the transmission to the sexual partners. In this paper we analyzed the genital HPV infection, some risk factors and the concordance among sexual couples. A cross-sectional study was done in 351 men and 28 sexual couples from southern Mexico. HPV infection was done by PCR with MY09/11 primers and genotyping with restriction fragment length polymorphism. HPV was detected in 231 men. The sexual age of onset, number sexual partners and history of extra marital sexual relations were not significantly associated with the HPV infection. HPV6 (34.2%), 16 (14.72%), 11 (11.69%), 62 (6.49%), and 81 (4.76%) were the most frequently detected HPV genotypes. High risk HPV prevalence was higher in the older men than in the youngest men and the concordance of viral genotypes between sexual couples was found in 28.6%. In conclusion, our study contributes to regional knowledge on HPV types, its distribution in different age groups and concordance of HPV types in sexual couples.

Keywords: HPV in men, viral types, concordance, sexual couples

Introduction

The human papillomavirus (HPV) is one of the most common sexually transmitted infections [1]. The World Health Organization estimates that the prevalence is about 630 million people. High risk (HR) HPV genotypes (HPV16 and 18) have a main role in the etiology of penile, oropharyngeal and anal cancers among men [2, 3]. Low risk (LR) HPV genotypes (HPV6 and 11) are associated with anogenital warts [4]. Genital warts often spontaneously resolve and are not life-threatening, nevertheless have high infectivity and risk of recurrence [5].

The prevalence of genital HPV infection in men vary widely due to differences in the populations studied, sampling sites, HPV DNA detection method used, race, age, sexual orientation and circumcision. Based on review of the literature, the prevalence of genital HPV infection in men ranges from 1.3% to 72.9% [6]. The HPV prevalence among men in the of general popu-

lations has been estimated to be 12.4% and in the high risk population at 30.9% [7]. In a study conducted in China it was reported that frequency of LR-HPV infection is similar between men aged 18-35 years and men greater than 35 years of age, while the frequency of HR-HPV infection is higher in men at 35 years of age [8]. In the general population the HPV6, 16, 66, 11, 84, 58, 53 and 61 have been the variants most frequently detected in men [6, 9]. The most frequent risk factors for HPV infection in men are having a high number of sex partners before and during marriage, first sexual intercourse at a young age and high frequency of sexual intercourse [10]. Conversely, circumcision and condom use has been associated with reduced detection of HPV infection in men [6, 10, 11]. However, more data are needed to obtain a better understanding of associated risk factors to HPV infection in men.

Some studies have reported that if HPV is present in one partner, it is efficiently transmitted to

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Table 1. Sociodemographic and sexual conduct characteristics associated with HPV infection in men

	HPV-		HPV+		OR	CI	P
	n = 120	%	n = 231	%			
Age (years) ^a	35.8 ± 10.4		35.0 ± 9.9				
Range	15-78		17-63				
Smoking status							
No	67	58.8	163	71.2	1		
Yes	47	41.2	66	28.8	0.57	0.35-0.95	0.021
Alcohol consumption							
No	21	20.2	49	25	1		
Yes	83	79.8	147	75	0.76	0.43-1.39	0.348
Education (years)							
13 or more	63	59.4	127	64.1	1		
10-12	20	18.9	34	17.2	0.84	0.43-1.68	0.595
9	13	12.3	20	10.1	0.76	0.33-1.78	0.485
6	10	9.4	17	8.6	0.84	0.34-2.18	0.689
Marital status							
Married	26	70.3	108	69.7	1		
Cohabiting	1	2.7	9	5.8	2.16	0.27-98.5	0.406
Single, never married	10	27.0	38	24.5	0.91	0.38-2.31	0.831
Sexual age of onset							
>20	11	9.7	30	13.0	1		
16-20	69	60.5	126	54.8	0.66	0.28-1.48	0.292
<16	34	29.8	74	32.2	0.79	0.32-1.88	0.580
No. of lifetime sexual partners							
1	18	15.8	48	20.9	1		
2-4	33	28.9	60	26.1	0.68	0.32-1.42	0.274
5-10	43	37.7	78	33.9	0.68	0.33-1.37	0.249
>11	20	17.6	44	19.1	0.82	0.36-1.88	0.618
History of sexual relations with prostitutes							
No	54	52.4	127	66.1	1		
Yes	49	47.6	65	33.9	0.56	0.33-0.94	0.021
History of sexual relations extra marital							
No	43	41.7	98	51.1	1		
Yes	60	58.3	94	48.9	0.68	0.41-1.14	0.127

HPV human papillomavirus, OR odds ratio, CI confidence interval; ^aExpressed as mean ± standard deviation; Significant differences are indicated in bold.

the other. The concordance of HPV genotypes among sexual couples has been evaluated in previous studies, although with marked variations in the concordance. In this regard, some studies conclude that concordance of HPV genotypes and of its prevalence among sexual partners are lower than expected.

Therefore in this paper we analyzed genital HPV infection, some risk factors and the concordance among sexual couples in one population located in southern Mexico.

Materials and methods

Study population

Men enrolled from 2009 to 2011 in the State of Guerrero located in southern Mexico. The participants were recruited of main public Health Centers and private medical offices, who attended for evaluation of penile related disease or to have a complete sexually transmitted diseases evaluation. 28 women, the sexual couples of men who were participants in this

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Table 2. Distribution of HPV genotypes in men

Genotypes	n	%	Genotypes	n	%	Genotypes	n	%
LR-HPV	153	66.23	HR-HPV	65	28.14	UR-HPV	13	5.63
6	79	34.20	16	34	14.72	26	3	1.30
11	27	11.69	18	3	1.30	53	5	2.16
13	1	0.43	31	3	1.30	70	4	1.73
40	2	0.87	35	1	0.43	73	1	0.43
54	2	0.87	39	1	0.43			
61	8	3.46	45	8	3.46			
62	15	6.49	52	2	0.87			
71	2	0.87	58	3	1.30			
81	11	4.76	59	2	0.87			
84	4	1.73	66	8	3.46			
86	1	0.43						
102	1	0.43						

LR low risk, HR high risk, UR undetermined risk.

study, accepted to participate and be evaluated for HPV infection. The men who participated were asked questions about sociodemographic characteristics and risk factors for HPV infection. The study was approved by the Institutional Ethics Committee and informed consent was obtained from all participants.

Sample collection

The epithelial cells from the urinary meatus (2 cm), balano-preputial lamina, frenulum of prepuce and gland of penis were obtained using a cytobrush, this was cut and placed in a tube containing lysis buffer (10 mM Tris pH 8.0, 20 mM EDTA pH 8.0 and 0.5% sodium dodecyl sulfate) and stored immediately at 4°C. For women, cervical scrapes were collected by sampling the ectocervix and endocervix with a cytobrush and cervical scrapes were also placed in a tube containing lysis buffer. The genomic DNA was extracted from epithelial cells using a standard phenol chloroform method [12].

HPV detection and genotyping

Purified DNA was amplified by PCR for a conserved 450 bp region of the HPV L1 gene with the primers MY09 and MY11 [13, 14]. The reaction mixtures (50 µl) contained 0.8 µM of each primer, 25 µl of AmpliTaq Gold® 360 PCR Master Mix (Applied Biosystems, Foster City, CA) and ~500 ng of target DNA. The reaction was done in GeneAmp PCR System 2400 (Applied Biosystems, Foster City, CA), under the

following conditions: 95°C, 10 min; 40 amplification cycles (95°C, 1 min; 58°C, 1 min and 72°C, 1 min) and 72°C, 10 min. The integrity of DNA samples was verified by amplification of a 268 bp region of the human β-globin gene using PCO4 and GH20 primers [15]. An HPV16 plasmid and genomic DNA from CaSki and HeLa cells were used as positive controls, and human DNA without HPV DNA and water were used as negative controls. For HPV genotyping, amplified PCR products were digested with restriction enzymes *Bam*HI,

*Dde*I, *Hae*III, *Hin*fI, *Pst*I, *Rsa*I and *Sau*3AI (Invitrogen, Carlsbad, CA) and RFLP analysis was performed to identify more than 40 genital types of HPV [16].

Statistical analysis

The results are expressed as the mean ± standard deviation. The association of potential determinants of HPV infection were estimated by odds ratios (OR) and 95% confidence intervals (CI). The Chi squared test (χ^2) was used to determinate the differences between HR or LR-HPV infection and age groups. *P* value was considered significant when < 0.05. Statistical analysis was performed using the STATA 10.0 software package (Stat Corporation, College Station, TX, USA).

Results

DNA from 120 HPV negative men and 231 HPV positive men were included in this study. The mean age was 35.8 ± 10.4 years old (range, 15-78) for HPV negative and 35.0 ± 9.9 years old (range, 17-63) for HPV positive. The main sociodemographic and sexual conduct characteristic associated with HPV infection are shown in **Table 1**. Alcohol consumption, education level, marital status, sexual age of onset, number sexual partners and history of extra marital sexual relations were not significantly associated with HPV infection in men. The smoking status and history of sexual relations with prostitutes were associated with a significantly decreased risk of HPV infection (OR 0.57,

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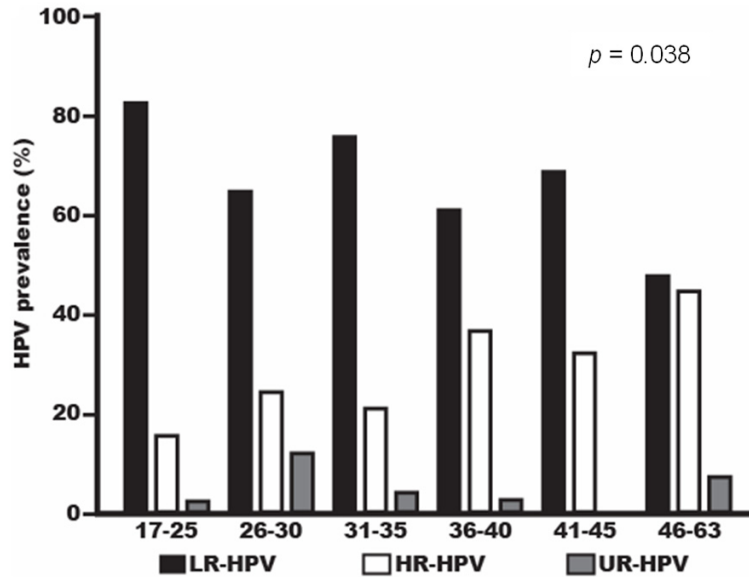


Figure 1. Prevalence of HPV by age group in men. LR low risk, HR high risk, UR undetermined risk. p value was calculated with χ^2 .

CI 0.35-0.95 and OR 0.56, CI 0.33-0.94, respectively). The distribution of HPV genotypes detected among the 231 men is shown in **Table 2**. LR-HPV genotypes were detected in 66.23%, HR-HPV in 28.14% and UR-HPV in 5.63% of the analyzed samples. HPV6 (34.2%), 16 (14.72%), 11 (11.69%), 62 (6.49%), and 81 (4.76%) were the most frequently detected HPV genotypes. The HPV prevalence by age group is shown in **Figure 1**. The highest LR-HPV prevalence was found in the 17-25 year age group and lowest prevalence in the 46-63 year age group, while the highest HR-HPV prevalence was found in the 46-63 year age group and lowest prevalence in the 17-25 year age group ($P = 0.038$). Cervical DNA from 28 women, the couples of 28 men included in this study was tested for HPV infection. The concordance of viral genotypes between 28 sexual couples is shown in **Table 3**. Total concordance was observed in 28.6%, partial concordance in 3.6% and it was absent in 67.8%.

Discussion

In this study, we analyzed genital HPV infection and some risk factors for infection in 351 men as well as the concordance of viral genotypes in 28 sexual couples.

In this work, the alcohol consumption, having few years of education, marital status, the sex-

ual age of onset, number of lifetime sexual partners and history of extra marital sexual relations were not associated with risk of genital HPV infection. Interestingly, smoking and have history of sexual relations with prostitutes were associated with a significantly decreased risk of HPV infection. In contrast, a previous study reported that smoking ≥ 10 cigarettes per day and lifetime number of female sex partners are risk factors for genital HPV infection in men [11]. In one study conducted in Brazil, United States and Mexico found the lifetime and recent number of sexual partners and having sex in the past 3 months as factors significantly associated with HPV

infection [17]. In addition, the presence of *C. trachomatis*, *N. gonorrhoea* was risk factors strongly associated with HPV infection in a study carried out in Kenya [18]. Therefore, it is clear that differences exist in the risk factors associated with HPV infection in men. These differences can possibly be due to differences in sample sizes, studied population, race, age and method of HPV diagnostic.

Several studies have reported that HPV genotypes 6 and 16 are the genotypes most commonly detected in men [7, 9, 19]. In agreement with previous results, we detected genotypes 11 and 16 as the most common genotypes found in men. The men with genital HPV infection act as a reservoir for women's infection [20]. Therefore is important to know the HPV genotypes circulating in the men, as one possible way to control and monitor the HPV infection in women. In fact, in the State of Guerrero located in southern Mexico (the place where this study was conducted) the cervical cancer and the cervical infection with genotypes 16, 18, 31, 33, 45, 6 and 11 are commonly identified [21, 22].

Previously published results regarding the age and HPV infection with high or low risk are contradictory. For example, Svare et al. [23] reported that younger age was associated with LR-HPV infection, whereas Baldwin et al. [24] report no association with age. In our study, we

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Table 3. Concordance of HPV genotypes between 28 sexual couples

Concordance	n	%	HPV genotype	
			Men	Woman
	1	3.6	81	81
	3	10.7	6	6
	3	10.7	16	16
	1	3.6	62	62
Total	8	28.6		
	1	3.6	16	6,16
Partial	1	3.6		
	1	3.6	16	45
	1	3.6	102	54
	1	3.6	6	66
	1	3.6	6	16
	1	3.6	11	16,62,45
	1	3.6	58	16
	1	3.6	62	53
	1	3.6	81	58
	1	3.6	11	66
	1	3.6	16	6
	1	3.6	11	34
	1	3.6	66	33,18
	1	3.6	84	81
	1	3.6	62	54
	1	3.6	11	18
	1	3.6	81	CP8304
	1	3.6	6	53
	1	3.6	6	Neg
	1	3.6	16	Neg
Absent	19	67.8		

found that the frequency of LR-HPV infection tends to decrease with age, whereas the frequency of HR-HPV infection tends to increase with age. In fact, in women from the State of Guerrero Mexico it was reported that the prevalence of HR-HPV infection increase with age [21]. Similarly, in Italy, similarities were found with respect to the associated factors and types of HPV in women and men of the same geographic region [9].

Several studies have analyzed the type-specific concordance between heterosexual couples [8, 19, 25]. We found total concordance in 28.6% of couples, frequency similar to those obtained by Hippelainen et al [26]. These results are in agreement with the hypothesis of sexual transmission of HPV infections [27]. In our study, the concordance was absent in 67.8%. This may be

explained by the difference in the time required for clearance of HPV infection in men and women, immune response and in general the natural history of HPV infection may be different between men and women due to differences between the epithelium in the cervical transformation zone and the penis [6, 10, 20].

In conclusion, our study contributes to regional knowledge on HPV types, its distribution in different age groups and concordance of HPV types in sexual couples. In addition, men with HR-HPV infection are important because men act as a reservoir for women's infection. Therefore, HPV infection in men affects disease risk in women. However, our study is smaller in scale compared with previous reports; therefore it's necessary to increase the size of the sample to obtain conclusive results.

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Disclosure of conflict of interest

None.

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