Research Article

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HPV in Female Partners Increases Risk of Incident HPV Infection Acquisition in Heterosexual Men in Rural Central Mexico

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Abstract

Background: Knowledge about the incidence, clearance, and factors associated with human papilloma virus (HPV) infection in men is lacking, and much of what is available focuses on high-risk groups.

Methods: A prospective cohort study was carried out with 504 heterosexual males from Mexico State, Mexico. Participants were monitored for 4 years at intervals of approximately 4 months, with a median total duration of follow-up of 19.8 months. The presence of cervical HPV in female sexual partners was evaluated as a risk factor. HPV DNA testing was conducted using the polymerase chain reaction technique. Risk factors associated with the incidence and clearance of HPV infection were evaluated through survival analysis.

Results: The cumulative incidence of infection with any HPV type at 12 months was 15% [95% confidence interval (CI), 0.12–0.20]. About 72% of men with incident HPV cleared the infection by 12 months. The presence of cervical HPV in a man's female sex partner was the main determinant for males acquiring HPV infection of any type [adjusted HR (AHR), 2.1; 95% CI, 1.1–3.8] and oncogenic HPV infection (AHR, 4.1; 95% CI, 2.1–8.0), while having a history of anal sexual relations with women was statistically associated with acquiring nononcogenic HPV (AHR, 1.8; 95% CI, 1.1–2.9).

Conclusions and Impact: The incidence of genital HPV infection in this population is relatively low, with relatively quick acquisition and clearance rates. Cervical HPV infection in men's female sexual partners was the main risk factor for genital HPV infection in men. *Cancer Epidemiol Biomarkers Prev; 21(11); 1956–65. ©2012 AACR.*

Introduction

Human papilloma virus (HPV) infection is the most common sexually transmitted infection among adolescents and adults (1). It is well established that HPV causes many life-threatening diseases in men and women, including cervical, vulvar, and vaginal intraepithelial neoplasia and invasive cancers in women (2, 3), oropharyngeal and anal cancer in either sex, and penile cancers in men (4, 5). It has been shown that male sexual behavior affects the risk of HPV infection, dysplasia, and cervical cancer in female partners, even after adjusting for female sexual behavior (6). Thus, a better understanding of HPV infection in men is an essential component of prevention programs aimed at reducing cervical cancer and other HPV-related diseases. However, little is known about the natural history of HPV infection in men.

To our knowledge, there are 9 documented scientific articles on 8 prospective studies on HPV infection in men (7–15). All of them provide information on incidence rates and 8 describe the persistence or clearance of HPV (7–11, 13–15). Twenty studies on risk factors for HPV infection in men are currently available, many of which are cross-sectional and only 7 are cohort studies (7, 9–12, 14, 15). Most of these studies have been restricted to high-risk populations, such as subjects who sought medical care at an sexually transmitted diseases (STD) clinic, military recruits, or male partners of women with HPV-related diseases.

The purpose of the present study is to estimate incidence and clearance rates of type-specific HPV infections and to evaluate the factors associated with infection, in heterosexual males with predominantly monogamous sexual behavior, living in a rural area in central Mexico. This should significantly extend knowledge on the natural history of HPV infection in men. It can also shed light on whether women's sexual behavior influences male partners' HPV risk.

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Materials and Methods

Study design and population

A prospective study of the natural history of HPV was carried out with 504 self-identified heterosexual males residing in 4 municipalities belonging to the Texcoco Sanitary District in the State of Mexico, in central Mexico. Men were invited to participate through their regular sexual partners, who agreed to participate in an HPV study of heterosexual couples who seek health services at public clinics open to the general population (Ministry of Health, Primary level health care; 16). The recruitment period was from January 23, 2003 to June 8, 2004. The participation criteria were: (i) being a permanent resident of the municipalities of Atenco, Tepetlauxtoc, Texcoco, or Chimalhuacan; (ii) being sexually active; (iii) agreeing to sign the informed consent form; (iv) committing to follow-up testing for 3 years; (v) being willing to provide information on sexual characteristics and behavior; and (vi) being in good health. Men who agreed to participate were scheduled for follow-up visits approximately every 4 months, for a total of 5 visits. This study includes 443 men who had at least 2 visits. During the first visit, men filled out a self-administered questionnaire, in complete privacy, which collected data on socioeconomic variables, educational level, smoking, circumcision status, and sexual behavior variables. Information about the presence of cervical HPV in female sexual partners was also included. During the baseline and follow-up visits a physical examination was conducted on the men, and samples of exfoliated genital cells were obtained for HPV DNA testing with PCR. A baseline sample of cervical epithelial cells was obtained from the female sexual partners. This study was approved by the ethics and research committees of the participating institutions.

In men, genital specimen collection was conducted as described elsewhere (17). Briefly, 3 samples of exfoliated epithelial cells were taken from external genitalia. The first was taken from the middle third of the scrotum and penis shaft, the second from the balano-preputial groove, and the third from the urinary meatus. A nylon brush and a Dacron swab were used for epithelial cell collection. The 3 samples were combined into one single tube and stored.

In women, a sample of epithelial cells was taken from the exocervix and endocervical canal using a nylon cytobrush, which was rotated 360°C to assure sampling of the cervical transformation zone. All brushes containing the collected material were placed in a 5 mL aliquot of PBS/merthiolate 0.01% (v/v) for storage. All samples were stored at −20°C before DNA extraction.

HPV DNA extraction

Before DNA extraction all samples were centrifuged at 4,500 rpm for 6 minutes. Precipitated matter was suspended in 1 mL 0.01 mol/L TRIS HCL pH 7.4. Genital samples were treated with proteinase K (170 μg/mL). DNA extraction was done with phenol-chloroform-isoamyl alcohol (24:1). Then 5 mol/L NaCl were added and precipitation was carried out with isopropanol. Finally, the supernatant was suspended in 50 μL TE buffer pH 7.6 and stored at −70°C (18).

HPV DNA detection and genotyping

The presence of HPV DNA was determined by DNA hybridization tests as described by Gravitt and colleagues (19). Before sample digestion, HPV DNA amplification and determination of β-globin was carried out in separate reactions. HPV DNA was amplified using biotinylated primers known as PG/MY L1. To determine specimen adequacy, a fragment of human gene from β-globin was coamplified with primers BGH20 and BPC04.

HPV detection and genotyping was done on PCR products (reverse hybridization), using nylon strips upon which hybridization was conducted. Each strip contained 39 test lines, 37 of which corresponded to type-specific tests for HPV and 2 of which quantify the low or high concentration of ß-globin. HPV types considered as high risk included in the test were 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 66, and low risk HPV types included were 6, 11, 26, 40, 42, 53, 54, 55, 61, 62, 64, 67, 68, 69, 70, 71, 72, 73, 81, 82, 83, 84, IS39, and CP6108 (20). Finally, hybridization bands were detected by calorimetry. Strips were interpreted using an acetate overlay that indicated the position of every specific HPV type. The result of hybridization was interpreted independently by 2 reviewers. ß-globin-negative samples with HPV-positive PCR were considered positive for the present analysis, as ß-globin may not be detected because of competition among oligonucleotides on the strips and PCR primers.

Statistical analysis

We evaluated the sociodemographic and sexual behavior characteristics of study participants at the time of recruitment. These characteristics were compared between subjects who were only evaluated at the baseline measurement and those that were also evaluated at the follow-up measurements, using Pearson χ^2.

Baseline prevalence (16), incidence rates, and clearance rates were estimated for any HPV type, oncogenic types, nononcogenic types, HPV 16 and/or 18, and specific HPV types. In the present analysis, single or multiple infection with oncogenic HPV or mixed infection with both types (oncogenic and nononcogenic) was classified as infection with oncogenic HPV. Single or multiple infection with only nononcogenic HPV types was classified as nononcogenic infection.

HPV incidence was estimated in men who were HPV negative at the beginning of the study and subsequently tested positive for one or more HPV types, and men who were positive for a specific HPV type at the beginning of the study and acquired one or more new HPV genotypes during follow-up.

In our calculation of incidence rates for HPV of any type and by risk group (oncogenic HPV and nononcogenic HPV), we included only men who tested negative for HPV at baseline and later became positive for HPV of the

corresponding group. Genotype-specific incidence rates were estimated based on the number of participants with incident infections; for each genotype, the population at risk was men without said genotype at the baseline measurement.

Confidence intervals (CI) of 95% were calculated for an incidence or clearance rate based on the number of events modeled as a Poisson variable divided by the total number of person–months (21, 22). HPV incidence and clearance rates per 1000 person-months were estimated assuming that HPV infections were acquired or cleared at the midpoint of the follow-up interval when the new infection was detected or cleared.

We used Nelson-Aalen and Kaplan Meier estimators to estimate the accumulated incidence of any type of HPV infection, oncogenic HPV, and nononcogenic HPV (23). Association patterns between HPV presence in the sex partner and HPV incidence in men were evaluated using the Kaplan–Meier test. Differences between groups were assessed using Wilcoxon and log-rank tests. Time at risk was determined as the number of months passed since the baseline measurement until the midpoint of the interval between the last infection-free date and the date when the new HPV infection was detected. Subjects who were free of HPV infection until the end of follow-up were censored at the date of the last follow-up.

The clearance analysis included those men with incident HPV types with at least one follow-up measurement after the detection of incident HPV. Clearance was expressed as the proportion of men with incident HPV that was negative to said genotype or risk group on one sequential follow-up visit and as the clearance rate per 1,000 person–months/observation after detection of HPV. Clearance time of HPV infection was defined as the time passed between the date at the midpoint of the interval between the last negative measurement and the first positive HPV result, and the midpoint of the interval between the last visit with a positive result and the first sequential visit with a negative HPV result. Participants whose infection did not clear were censored at the date of the last positive HPV test result. The duration of infection was estimated using the Kaplan–Meier method.

We evaluated the effect of potential risk factors on HPV acquisition and clearance rate using Cox proportional hazards models. The risk of acquiring HPV infection was estimated for any HPV type, oncogenic HPV types, and nononcogenic HPV types. This analysis evaluated sexual behavior and sociodemographic characteristics measured at the beginning of the study and cervical HPV in female partner as potential risk factors. The variables that were found to be associated with a P value <0.1 in the univariate analysis were included in the multivariate models. Univariate predictors not included in the model were evaluated as confounders. If a 15% or greater change was observed in any of the estimated effects, variables were included in the model. The measure of association was the HR with 95% CI. Assessment of model fit was examined, including verification of the proportional hazards assumption.

Incidence rates and specific infection duration by age group were estimated for any type HPV, oncogenic HPV, and nononcogenic HPV. Differences in HPV incidence by age group were evaluated based on CIs derived from the Poisson distribution. Differences in HPV infection clearance by age groups were evaluated using the log-rank test.

The statistical analysis was conducted using Stata 9.0 statistical software (Stata Corporation).

Results

Out of 504 subjects who agreed to participate in the study, 103 (20.4%) were positive for HPV of any type at the baseline measurement (data not shown). Of the remaining 401 subjects, 351 underwent at least one follow-up measurement. The median duration of follow-up for these subjects was 19.77 months (inter-quartile range (IQR), 13.1–25.8 months], with a median duration of 3.7 months between follow-up visits.

Table 1 shows the distribution of sociodemographic and sexual behavior characteristics in the participants included in this analysis of baseline HPV negative men. At baseline the median age of the 401 HPV negative men was 36 years (IQR, 30–44 years), the median age at which they initiated sex was 18 years (IQR, 17–20), and their median number of lifetime sex partners was 1 (IQR, 1–2). Table 1 shows the distribution of baseline characteristics in men negative at baseline for HPV with at least one follow-up measurement compared with those with no follow-up measurements. Among men with follow-up measurements there was a greater proportion of men aged 31 to 40 ($P = 0.042$), living in a rural area ($P = 0.003$), and who were married ($P = 0.002$) compared with baseline HPV negative men with no follow-up. Out of the 351 subjects with follow-up measurements, 53% first had sex at age 18 or younger, 52.4% had 1 to 2 sex partners, 80.9% had not had sexual relations with prostitutes, 62.9% had not had anal sexual relations, and 93.2% were not circumcised.

Out of the 504 subjects that agreed to participate in the study, the population of men at risk for HPV of any type at baseline with at least one follow-up measurement consisted of 351 subjects; for oncogenic HPV it was 405 subjects; for nononcogenic HPV it was 376 subjects; and the maximum population at risk for specific HPV types was 443 subjects. During follow-ups, out of this population at risk we observed 84 subjects with incident any type HPV infections, 47 with incident oncogenic HPV infection and 69 with nononcogenic HPV, out of which 42 any type HPV infections were cleared, as well as 22 oncogenic HPV and 38 nononcogenic HPV infections (data not shown).

Incidence rates and duration of HPV infection are presented in Fig. 1. The estimated incidence rate for HPV infection of any type was 12.3/1,000 person–months (95% CI, 9.8–15.2). The incidence rate for oncogenic HPV types was 5.6/1,000 person–months (95% CI, 4.1–7.4), and for

Table 1. Distribution of sociodemographic and sexual behavior characteristics in heterosexual men with measurements taken only at the baseline and in men who provided follow-up measurements

Characteristics	Males negative for any HPV type at the baseline measurement $n = 401$ *n* (%)	Males negative for any HPV type at the baseline, with no follow-up measurements $n = 50$ *n* (%)	Males negative for any HPV type at the baseline, with at least one follow-up measurement $n = 351$ *n* (%)	*P*[a]
Age (y)				
18–24	31 (7.7)	8 (16.0)	23 (6.5)	0.042
25–30	74 (18.5)	12 (24.0)	62 (17.7)	
31–40	162 (40.4)	14 (28.0)	148 (42.2)	
41–75	134 (33.4)	16 (32.0)	118 (33.6)	
Place of residence				
Rural	288 (71.8)	27 (54.0)	261 (74.4)	0.003
Urban	113 (28.2)	23 (46.0)	90 (25.6)	
Socioeconomic level				
Low	133 (33.6)	10 (20.4)	123 (35.4)	0.111
Medium	132 (33.3)	19 (38.8)	113 (32.6)	
High	131 (33.1)	20 (40.8)	111 (32.0)	
Marital status				
Married	328 (81.8)	33 (66.0)	295 (84.1)	0.002
Single	73 (18.2)	17 (34.0)	56 (15.9)	
Educational level				
≤6 years	127 (31.7)	16 (32.0)	111 (31.6)	0.736
7–9 years	162 (40.4)	18 (36.0)	144 (41.0)	
≥10 years	112 (27.9)	16 (32.0)	96 (27.4)	
Religion				
Catholic	349 (87.0)	43 (86.0)	306 (87.2)	0.816
Other	52 (13.0)	7 (14.0)	45 (12.8)	
Current smoking				
No	222 (55.4)	22 (44.0)	200 (57.0)	0.084
Yes	179 (44.6)	28 (56.0)	151 (43.0)	
Age at sexual initiation				
≤18 years	216 (53.9)	30 (60.0)	186 (53.0)	0.352
≥19 years	185 (46.1)	20 (40.0)	165 (47.0)	
Lifetime female sexual partners				
1	155 (38.7)	19 (38.0)	136 (38.7)	0.415
2	59 (14.7)	11 (22.0)	48 (13.7)	
3 to 9	140 (34.9)	14 (28.0)	126 (35.9)	
10 or more	47 (11.7)	6 (12.0)	41 (11.7)	
Reported ever having insertive anal sex with female				
No	241 (62.6)	29 (60.4)	212 (62.9)	0.739
Yes	144 (37.4)	19 (39.6)	125 (37.1)	
Circumcision				
No	371 (92.5)	44 (88.0)	327 (93.2)	0.194
Yes	30 (7.5)	6 (12.0)	24 (6.8)	
History of sexual relations with prostitutes				
No	323 (80.5)	39 (78.0)	284 (80.9)	0.627
Yes	78 (19.5)	11 (22.0)	67 (19.1)	
Condom use when having anal sexual relations with prostitutes				
Has never had sexual relations with prostitutes	323 (80.5)	39 (78.0)	284 (80.9)	0.790
Always	26 (6.5)	3 (6.0)	23 (6.6)	
Not always	52 (13.0)	8 (16.0)	44 (12.5)	

[a] ρ value obtained by χ^2 test.

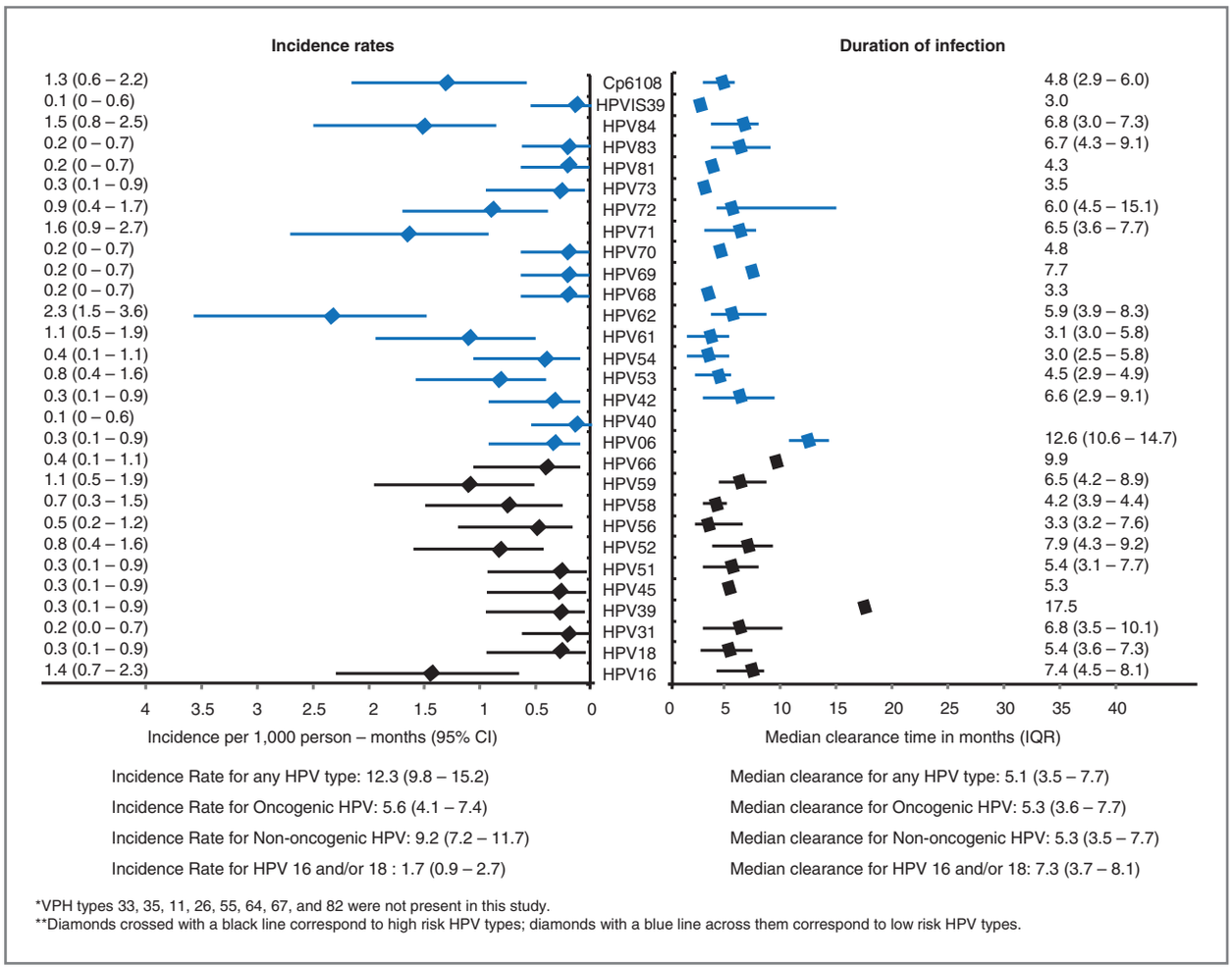

Figure 1. HPV incidence rates and median clearance time by specific HPV type in a group of heterosexual males in rural Mexico State.

nononcogenic HPV it was 9.2/1,000 person–months (95% CI, 7.2–11.7). The highest type-specific oncogenic HPV incidence rates were for HPV 16, 59, 52, and 58. Conversely, for nononcogenic HPV types the highest rates were found for HPV 62, 71, 84, and Cp6108.

The median clearance time for HPV infection of any type (defined as the time during which 50% of infections are cleared) was 5.1 months (IQR, 3.5–7.7). The duration of infection was similar for oncogenic and nononcogenic HPV types. The median time until oncogenic HPV clearance was 5.3 months (IQR, 3.6–7.7) and for nononcogenic HPV types 5.3 months (IQR, 3.5–7.7). For the oncogenic HPV types, median time to clearance was longest for HPV types 52, 16, and 31. For the nononcogenic HPV types, median time to clearance was longest for HPV types 6, 84, and 83 (Fig. 1).

The cumulative incidence of HPV infection and the time to clearance of infection among men are presented in Fig. 2. The cumulative incidence of infection for any HPV type at 12 months was 15% and at 40 months it was 31%. The proportion of the population that acquired nononcogenic HPV at 12 months was 12%, whereas only 7% acquired oncogenic HPV. Figure 2 shows that after 6 months, approximately 60%, 59%, and 58% of men with HPV remained positive for any HPV type, oncogenic HPV, and nononcogenic HPV, respectively, while after 12 months 28%, 22%, and 29% remained positive for these.

Factors associated with HPV infection and clearance

Table 2 shows the association observed between specific sociodemographic and sexual behavior variables of interest with the acquisition of any type, oncogenic, and nononcogenic HPV infection in univariate and multivariate models. An important increase was observed in the risk of acquiring a new HPV infection of any type in men whose sexual partners have cervical HPV. This association was also present in the analysis adjusted by male's current age, marital status, smoking, age of sexual initiation, history of anal sexual relations, and circumcision status (HR, 2.1; 95% CI, 1.1–3.8). Similarly, in the univariate and multivariate analysis we observed that the presence of any type cervical HPV infection in the female sex partner

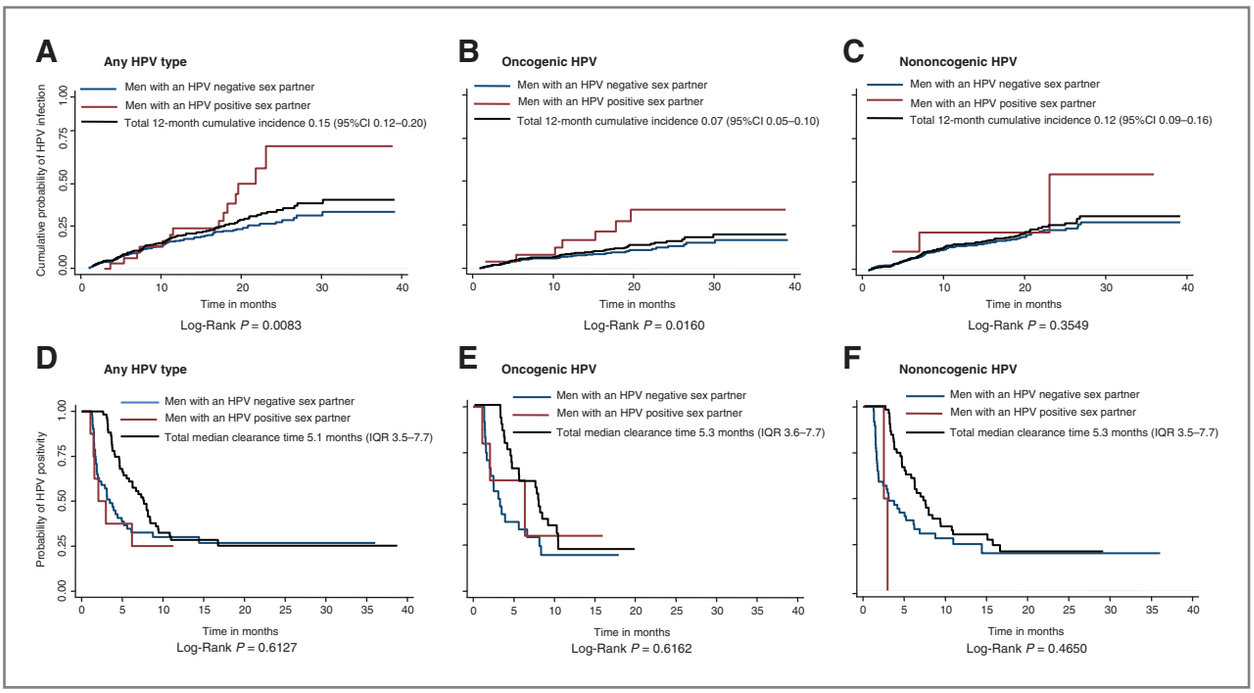

Figure 2. Nelson–Aalen cumulative risk estimators (A, B, C) and Kaplan–Meier clearance estimators and median clearance time (t_{50}; D, E, F) for any HPV type, oncogenic, and nononcogenic HPV infection for males with and without an HPV positive sex partner.

was significantly associated with oncogenic HPV acquisition in the male (HR, 4.1; 95% CI, 2.1–8.0), although this association was not observed for nononcogenic HPV. Having a history of anal sexual relations was statistically associated with nononcogenic HPV acquisition [adjusted HR (AHR), 1.8; 95% CI, 1.1–2.9]. These associations were constant even after adjusting for other factors, including socioeconomic level, religion, number of lifetime sexual partners, and condom use (data not shown). Figure 2 shows that men with HPV positive partners were at greater risk of infection with any type HPV and oncogenic HPV, compared with men with HPV negative partners (log-rank $P = 0.008$ and log-rank $P = 0.016$, respectively). The presence of HPV in female sexual partners was not associated with the clearance time of the HPV infection in men.

None of the factors assessed were found to be associated with any type HPV, oncogenic HPV, or nononcogenic HPV clearance in the univariate or multivariate analyses (data not shown).

Figure 3 shows that there was no statistically significant difference between age groups in incidence rates and median HPV clearance time by oncogenic potential.

Discussion

Our study contributes to the currently scarce knowledge available on the natural history of HPV infection in heterosexual males. We evaluated the factors associated with acquisition and clearance of any, oncogenic and nononcogenic HPV infections, and found that the presence of cervical HPV of any type in men's female sex partners was significantly associated with male HPV infection.

About the natural history of HPV infection in men, we found that the cumulative incidence of HPV after 12 months in rural Mexican men was 15%, similar to the 13.4% at 12 months reported in Mexican military personnel and 13.8% at 8 months in Danish military personnel (10, 11). These estimates are lower than those observed in men in southern Arizona and in young male students of the University of Washington in Seattle, who had incidences of 29.2% and 62.4%, respectively (12, 13). A recent study by Giuliano and colleagues that included men aged 18 to 70 years living in Mexico, Brazil, and the United States also showed a high incidence (39.3%) of all types of HPV at 12 months (15).

Our findings about HPV clearance also fell in between highs and lows from prior studies. We found that 72% of incident HPV infections had cleared within 12 months. A Japanese study found that infection persisted in 87.5% of clinically healthy subjects after 30 days, whereas it persisted in 100% of urethritis patients after 3 months; it persisted in 0% after 6 months (9). In Danish military personnel persistent infection was found in 18.4% after 8 months (10). In Mexican soldiers this figure was 17.3% (11). Clearance of any type HPV infection was observed in 75% of men from southern Arizona 12 months after initial HPV detection (13).

In the aforementioned study by Giuliano and colleagues, the median clearance time for any HPV type was 5.9 months (13), while the prospective study of HPV in

Table 2. Sociodemographic and sexual behavior characteristics associated with HPV acquisition in heterosexual males in rural Mexico State

	Any type HPV		Oncogenic HPV		Non-oncogenic HPV	
Variable	Univariate HR (95% CI)	Multivariate[a] adjusted HR (95% CI)	Univariate HR (95% CI)	Multivariate[a] adjusted HR (95% CI)	Univariate HR (95% CI)	Multivariate[a] adjusted HR (95% CI)
Presence of HPV in the sex partner						
No	1.0	1.0	1.0	1.0	1.0	1.0
Yes	2.1 (1.2–3.8)	2.1 (1.1–3.8)	4.2 (2.3–7.7)	4.1 (2.1–8.0)	1.1 (0.5–2.2)	1.1 (0.5–2.2)
Age (y)						
18–24	1.0	1.0	1.0	1.0	1.0	1.0
25–30	1.1 (0.4–2.7)	1.2 (0.5–3.0)	0.7 (0.2–2.4)	0.6 (0.2–2.2)	1.4 (0.5–4.2)	1.4 (0.5–4.4)
31–40	0.7 (0.3–1.6)	0.7 (0.3–1.8)	0.6 (0.2–1.9)	0.7 (0.2–2.1)	0.8 (0.3–2.4)	0.9 (0.3–2.6)
41–75	1.1 (0.4–2.6)	1.2 (0.5–2.9)	0.8 (0.3–2.4)	0.7 (0.2–2.4)	1.6 (0.6–4.6)	1.7 (0.6–5.0)
Marital status						
Married	1.0	1.0	1.0	1.0	1.0	1.0
Single	1.01 (0.5, 1.7)	0.8 (0.4, 1.5)	1.01 (0.5, 2.1)	0.8 (0.3,1.7)	1.04 (0.6–1.9)	0.9 (0.5–1.7)
Current smoking						
No	1.0	1.0	1.0	1.0	1.0	1.0
Yes	1.04 (0.6–1.6)	0.9 (0.6–1.5)	1.3 (0.7–2.3)	1.2 (0.7–2.3)	1.02 (.6, 1.6)	0.9 (0.6,1.5)
Age at sexual initiation						
≤18 years	1.2 (0.8–1.9)	1.2 (0.7–1.9)	1.2 (0.7–2.2)	1.3 (0.7–2.4)	1.2 (0.8–2.0)	1.1 (0.7 -1.8)
≥19 years	1.0	1.0	1.0	1.0	1.0	1.0
Lifetime female sexual partners						
1	1.0	NA	1.0	NA	1.0	NA
2	1.5 (0.8–2.8)	NA	0.9 (0.4–2.5)	NA	1.4 (0.7–2.9)	NA
3 to 9	1.2 (0.7–1.9)	NA	1.1 (0.5–2.1)	NA	1.4 (0.8–2.4)	NA
10 or more	1.3 (0.6–2.6)	NA	1.4 (0.6–3.3)	NA	1.1 (0.5–2.5)	NA
Reported ever having insertive anal sex with female						
No	1.0	1.0	1.0	1.0	1.0	1.0
Yes	1.5 (0.9–2.3)	1.4 (1.0–2.2)	0.8 (0.4–1.5)	0.7 (0.3–1.3)	1.7 (1.1–2.8)	1.8 (1.1–2.9)
History of sexual relations with prostitutes						
No	1.0	NA	1.0	NA	1.0	NA
Yes	1.3 (0.8–2.2)	NA	1.4 (0.8–2.7)	NA	1.3 (0.8–2.3)	NA
Circumcision						
No	1.0	1.0	1.0	1.0	1.0	1.0
Yes	1.8 (0.9–3.5)	1.5 (0.7–3.0)	1.4 (0.5–3.8)	1.3 (0.4–3.7)	1.5 (0.6–3.4)	1.2 (0.5–2.7)

Abbreviation: NA, not applicable.

[a]Adjusted for presence of HPV in the sex partner, age, marital status, current smoking, age at sexual initiation, history of anal sexual relations, and circumcision.

men (HIM study) found that the median duration of any HPV type was 7.5 months (15). In our study this time period was 5.1 months. It is important to consider that the cited studies had different time ranges between measurements, that the number of follow-up measurements varied, and that each study had a different duration. Our study included subjects with a baseline measurement and 1 to 5 follow-up measurements, with approximately 4 months between measurements. Considering the differences between populations and sampling techniques used by these studies, it is difficult to compare their results.

To our knowledge, this is the first study that evaluates the presence of cervical HPV in the female sex partner as a variable associated with genital HPV incidence and clearance in the male. We found that presence of HPV in men's female sex partners was the main determining factor for any type HPV acquisition (AHR, 2.1; 95% CI, 1.1–3.8) and for oncogenic HPV (AHR, 4.1; 95% CI, 2.1–8.0). Prior studies have shown that the main risk factors for genital HPV infection in women are related not only to their own sexual behavior but also to their partners' behavior (6). Our study paves the way for considering the influence that female sexual behavior might have on HPV acquisition in women's male sex partners. This extends to nonvaginal sex, as we found that a history of anal sexual relations with women was significantly associated with acquiring nononcogenic HPV (AHR, 1.8; 95% CI, 1.1–2.9). This result is consistent with Lajous and collaborators' finding that the risk of acquiring HPV increases 5 times in men who have sexual anal relations with men (11), and with Moscicki and collaborators' report of a 7-fold increased risk of anal HPV infection in young

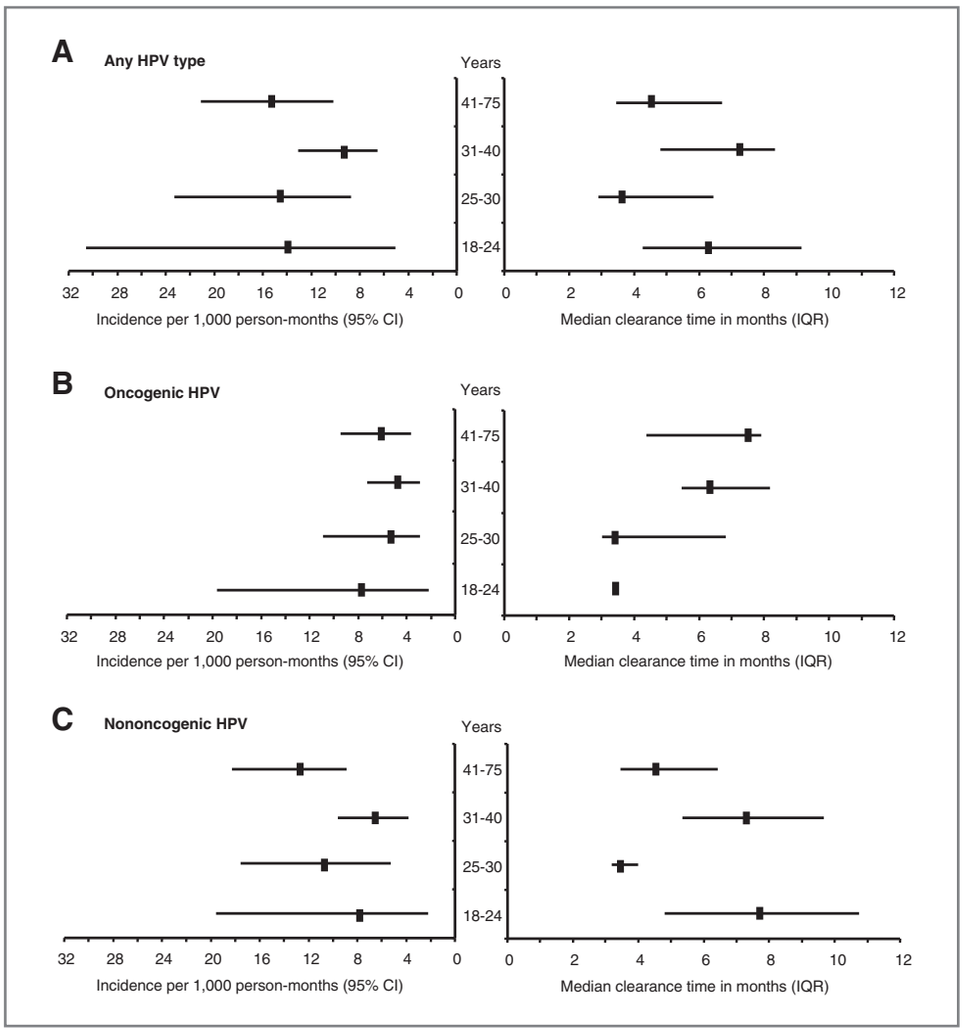

Figure 3. Incidence rates and median clearance time for HPV infection by oncogenic potential and by age groups in heterosexual males in rural Mexico state.

heterosexual women with a history of anal sexual relations (24). Other sexual behavior variables (age at sexual initiation, lifetime number of sex partners, number of sex partners before age 20, use of condoms), circumcision, smoking, and sociodemographic variables (age, marital status, socioeconomic level, education level, and religion) were not associated with any type HPV acquisition, oncogenic HPV, or nononcogenic HPV. Nor did we find any variables associated with HPV clearance in any of the different oncogenic potentials. Like Giuliano and collaborators (13, 15), we found no clear pattern was observed in HPV acquisition and clearance in the different HPV groups by age, as other studies have shown among women (25).

We found that the main predicting factors for HPV infection are sexual behavior variables. However, these associations have not been found in higher risk groups, which may be because of high baseline HPV infection rates in high-risk male populations and across all promiscuity levels (26). Another possible explanation for this lack of association may be that samples are relatively small or that participants do not give exact information on their sexual background or current practices, which can lead to misclassification bias of potential risk behaviors. Thus, the lack of association we found between sexual behavior variables and HPV incidence could be because of our inclusion of a population with relatively low-HPV rates across strata. However, nondifferential misclassification of sexual behaviors is also possible.

Also, similar to other studies we did not find the expected protective effect of circumcision and condom use on HPV acquisition and clearance. The protective effect may be limited as the effect of circumcision could be limited to certain sites of the penis; similarly, the protective effect of the condom would be associated with HPV in the sites covered by the condom (27). In our study we cannot differentiate among sites because samples taken from the scrotum and penis shaft, balano-preputial

groove, and urinary meatus were combined. As for the lack of association between condom use and HPV infection, it has been attributed to behavioral aspects, such as the difficulty in using a condom consistently in stable relationships, and to biologic aspects such as HPV infection beyond the anatomical area covered by the condom (28).

Tobacco use is considered an indicator of high-risk sexual conduct and has been identified as a risk factor for HPV detection in men (29), as well as persistent HPV infection and anal and penile cancer in men (4, 30). However, the results of studies that have evaluated relationships between the 2 have been inconsistent. In the present study we also failed to find an association between smoking and HPV incidence.

When interpreting our study results, it is important to account for its limitations. First, having approximately 4-month intervals, and longer intervals in some patients, between follow-up visits may result in underestimation of HPV acquisition. In the infection clearance analysis we included only incident infections, avoiding infection duration bias by including prevalent infections, as it was unknown how long infections had been present before men entered the study. Even so, clearance time estimates may be biased because these infections were followed up for only a short time and infection clearance may have escaped observation before the end of the study. Furthermore, because of the short follow-up time of incident infections, we could not confirm clearance with a subsequent negative result. Data on potential risk factors were obtained by self-report; however, as participants did not know their HPV status at the baseline measurement, differential misclassification that could bias the results is unlikely. Finally, although we tried to achieve a representative sample of heterosexual men from 4 municipalities in Mexico State, the self-selection involved in recruitment through couples resulted in inclusion of a greater proportion of men older than 30 years, married, and who resided in rural areas. Thus, the population's characteristics limit the generalizability of our findings.

Our study makes an important contribution to knowledge of the natural history of HPV infection in males in a low-risk population. However, more cohort studies are needed that include men with a wider age range, different risk profile, and with shorter follow-up intervals and longer overall follow-up periods.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Development of methodology: A. Cruz, E. Lazcano-Ponce
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): A. Cruz, E. Lazcano-Ponce
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): R. Morales, A.R. Giuliano, A. Cruz, J. Salmeron, E. Lazcano-Ponce, X. Castellsagué
Writing, review, and/or revision of the manuscript: R. Morales, A.R. Giuliano, A. Cruz, J. Salmeron, E. Lazcano-Ponce, X. Castellsagué
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): R. Parada, A. Cruz, J. Salmeron
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