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Association of *Chlamydia trachomatis* infection and herpes simplex virus type 2 serostatus with genital human papillomavirus infection in men: *the HIM Study*

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Abstract

Background—Studies in women indicate that some sexually transmitted infections promote human papillomavirus (HPV) persistence and carcinogenesis. Little is known about this association in men, therefore we assessed whether *Chlamydia trachomatis* (CT) infection and herpes simplex virus type 2 (HSV-2) serostatus are associated with genital HPV prevalence, an early event in HPV related pathogenesis.

Methods—Genital exfoliated cells, first-void urine and blood from 3,971 men recruited in the USA, Mexico, and Brazil, were tested for HPV, CT, and HSV-2 antibodies, respectively. Multivariable logistic regression was used to assess the association of CT infection and HSV-2 serostatus with four HPV outcomes (any, oncogenic, non-oncogenic only, and multiple infections).

Results—A total of 64 (1.6%) men were CT positive and 811 (20.4%) men were HSV-2 seropositive. After adjustment for potential confounders, CT was associated with any HPV (aOR 2.19, 95%CI: 1.13–4.24), oncogenic HPV (aOR 3.10, 95%CI: 1.53–6.28), and multiple HPV (aOR 3.43, 95%CI: 1.69–6.95) prevalence. HSV-2 serostatus was associated with any HPV (aOR 1.25, 95%CI: 1.02–1.52), non-oncogenic HPV only (aOR 1.38, 95%CI: 1.08–1.75), and multiple HPV (aOR 1.33, 95%CI: 1.06–1.68) prevalence. In analyses stratified by sexual behaviour, CT infection was significantly associated with HPV detection among men reporting 2 recent sexual

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partners, while HSV-2 serostatus was significantly associated with HPV detection in men reporting 0-5 lifetime sexual partners.

Conclusion—In this population, CT infection and HSV-2 serostatus were associated with prevalent genital HPV infection. Future prospective studies should investigate whether these infections influence HPV acquisition and/or persistence.

Keywords

Men; genital; sexually transmitted infection; Human papillomavirus; *Chlamydia trachomatis*; herpes simplex virus type 2

Introduction

Human papillomavirus (HPV) is a common sexually transmitted infection (STI) and the primary etiologic agent in the development of anogenital cancers and condylomas (1). Factors shown to enhance progression of infection to cancer in women are smoking, long-term oral contraceptive use, and high parity (2). STIs also have been investigated as possible cofactors because they may directly interact with HPV, or damage the epithelial barrier that helps protect against HPV infection (1-3). Studies in women indicate that *Chlamydia trachomatis* (CT), assessed by the presence of DNA (4;5), may increase the risk of HPV persistence and that CT (assessed by the presence of DNA (6) and antibodies (7-9)) increase the risk of cervical cancer, although some uncertainty remains (10-12). The association between herpes simplex virus type 2 (HSV-2) (detected by DNA (13;14) or antibodies (15;16)) and cervical cancer remains equivocal. Little is known about the influence of STIs on HPV natural history in men. One prospective study found current CT infection to increase the risk of acquiring an additional HPV type in HPV-positive men (17). Four cross-sectional studies showed that STIs were associated with current HPV infection (18-21), but not all studies adjusted fully for sexual behavior.

In a study among men (22), we examined the association of CT infection and HSV-2 serostatus with four outcomes: any HPV, oncogenic HPV, non-oncogenic HPV only, and multiple HPV infections, after adjustment for confounding factors. Furthermore, we assessed whether such associations remained after stratifying by sexual behavior.

Materials and Methods

Study population

The HPV in Men (HIM) study is an ongoing prospective study of the natural history of HPV infection in men. Participants were recruited from Sao Paulo (Brazil), Cuernavaca (Mexico), and Tampa (United States), from March 2005 through September 2009. Men were eligible for participation if they were 18 to 70 years of age, reported no history of anogenital cancer or anogenital warts, had not participated in an HPV vaccine study, reported no current penile discharge or dysuria, and were not being treated for an STI. A full description of the study methods and procedures has been published previously (22).

Data and sample collection

Participants provided written informed consent and underwent a clinical examination two weeks (baseline) before the enrollment visit. Participants completed a computer-assisted self-interview questionnaire to determine sociodemographic characteristics, sexual history, condom use practices, tobacco use, self and partner history of STI, and self and partner recent diagnosis of an STI or warts. Using three saline prewetted Dacron swabs, study clinicians collected exfoliated cells from the coronal sulcus, penile shaft, and scrotum to test

for HPV infection. Samples were combined into one genital external specimen and stored at -70°C until analysis. Blood samples were collected to test for HSV-2 serostatus and men provided a first-void urine specimen for CT infection testing. Baseline (visit 1) samples and study characteristics were used for analyses.

Laboratory methods

Detection of HPV DNA was done by means of PCR amplification and genotyping. DNA was extracted with the QIAamp Media MDx (QIAGEN, Valencia, CA, USA) by a robotic system according to the manufacturer's instructions. DNA was stored at -20°C until use. 30 ng of DNA was amplified with the PGMY09/11 *L1* consensus primer system (23). HPV DNA was detected by running the sample on a 2% agarose gel to visualize a band at 450 bp. The PCR product was used for HPV genotyping and analyzed by using the Linear Array method (Roche Molecular Diagnostics city, CA, USA) (24). HPV genotyping was conducted on all samples regardless of HPV PCR results.

Serum for HSV-2 testing was stored at 4°C and analyzed within 24 hours. Detection of HSV-2 IgG antibodies was conducted with the HerpeSelect2 ELISA IgG kit (Focus Diagnostics Cypress, CA, USA). Samples were considered positive if the OD was >1.1 times above the cut-off calibrator.

Participants' first-void urine samples were collected into urine collection cups free of preservatives. Samples were transferred within 24 hours after collection into the GenProbe specimen transport tube. CT was tested using the *Chlamydia* LCx assay (Abbott Laboratories, Abbott Park, Chicago, IL, USA). Samples were concentrated (target capture), amplified (Transcription-Mediated Amplification) and detected (Dual Kinetic Assay) according to manufacturer's procedures.

HPV classification

We considered four HPV categories in our analyses: any HPV, oncogenic HPV, non-oncogenic HPV only, and multiple HPV infections. A participant was considered HPV positive if he tested positive by PCR or tested positive for at least one genotype. The oncogenic HPV category included men who were positive for at least 1 of 13 oncogenic types (HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68) regardless of co-infection with non-oncogenic types. Non-oncogenic HPV only included single or multiple infections with non-oncogenic HPV types (HPV types 6, 11, 26, 40, 42, 53, 54, 55, 61, 62, 64, 66, 67, 69, 70, 71, 72, 73, 81, 82, 83, 84, IS39 and CP6108) in the absence of oncogenic infection (25). A participant was considered positive for multiple HPV types if he tested HPV positive for >1 genotype, regardless of type. Samples were considered valid if they were HPV positive by PCR or by genotyping, or β -globin positive, regardless of HPV result. In total, 98% (3,971/4,074) of samples were considered valid. In a sensitivity analysis, similar results were obtained for the full cohort (n=4,074) as for the group with valid HPV samples only (n=3,971).

Statistical analyses

Pearson's Chi-squared test was used to compare HPV prevalence with CT prevalence and HSV-2 seroprevalence. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using bivariable and multivariable logistic regression analyses. We constructed four separate models, one for each HPV outcome: any HPV, oncogenic HPV, non-oncogenic HPV only, and multiple HPV infections. Men who had no detectable HPV (HPV negative on both PCR and genotyping assays) served as the reference group for all analyses.

Multivariable logistic regression analyses were performed using three different approaches. In approach 1, we used the same set of variables for all models; these were variables that had previously been associated with genital HPV prevalence or acquisition (22;26). This resulted in a list of six possible confounders for the CT model: (1) age (four categories); (2) country of residence; (3) smoking status (never, former, current); (4) education, (5) number of female partners during the past three to six months; and (6) number of male anal sex partners during the past three months. For the HSV-2 model, we also selected possible confounders (1) through (4) and additionally adjusted for (5) number of *lifetime* female sex partners and (6) number of *lifetime* male anal sex partners. Recent number of sex partners was used with CT, and lifetime number of sex partners was used with HSV-2, as CT diagnosis indicates current infection, while HSV-2 antibody status reflects cumulative lifetime exposure.

In approach 2, a list of potentially confounding variables that were associated ($P<0.25$) with each of the four HPV outcomes in bivariable analyses was added to the above named model (e.g. marital status, circumcision status, age of sexual debut, ever diagnosed with an STI, condom use during vaginal or anal sex in the past 3-6 months, and partner diagnosed with genital warts or with an STI during the past 6 months).

In approach 3, all variables associated ($P<0.25$) with each of the four HPV outcomes in bivariable analyses were added to the main model and dropped one by one based on the likelihood ratio test at ($p<0.05$), until a parsimonious model was obtained.

To explore whether sexual behavior modified the association of CT infection and HSV-2 serostatus with the HPV outcomes, we performed a stratified analysis in which we divided the study population into three equal-size groups by number of recent sexual partners (RSP) during the past 3-6 months, and by lifetime number of sex partners (LSP), respectively. Stratification was performed by number of partners, regardless of the gender of the partner. Men who refused to answer the number of male or female partners were excluded from the stratified analyses. Additionally, stratified analyses by country were performed.

All statistical tests were two-sided and all analyses were performed using Stata software version 11.2 (Stata Intercooled, College Station, TX, USA).

Results

Participating men had a median age of 31 years (interquartile range: 23-40) and were recruited from Brazil (35%), Mexico (33%), and the United States (32%). Overall, 2,650 (67%) men were positive for any HPV, 1,183 (30%) positive for oncogenic HPV, 898 (23%) positive for non-oncogenic HPV only, and 1,146 (29%) positive for multiple HPV infections.

Population characteristics are presented by CT infection and HSV-2 serostatus in Table 1. In total, 64 (1.6%) men were infected with CT at baseline. CT infection was most common (2.4% CT-infected) in the youngest age group (18-30 years) ($P<0.001$). CT prevalence was highest in men recruited from Brazil (2.3%) followed by Mexico (1.3%) and the United States (1.2%) ($P=0.038$). A higher number of recent female sexual partners during the past three to six months ($P=0.026$), and having a partner diagnosed with genital warts during the past six months ($P=0.028$) were significantly associated with CT infection.

In total, 811 (20.4%) men had antibodies against HSV-2. HSV-2 seroprevalence increased with age ($P<0.001$), with men from Brazil having the highest HSV-2 seroprevalence (38.4%). HSV-2 seroprevalence was associated with marital status ($P<0.001$), education ($P<0.001$), smoking ($P=0.045$), and circumcision ($P<0.001$). HSV-2 seroprevalence was

highest in men who reported ≥20 lifetime female sexual partners ($P<0.001$) and increased with lifetime number of male anal sexual partners ($P<0.001$). All other sexual behavior variables were associated with HSV-2 serostatus, except condom use during vaginal or anal sex during the past 3-6 months and number of recent female sexual partners.

Table 2 shows the prevalence of HPV infection by CT infection status and HSV-2 serostatus. HPV prevalence was higher in CT-infected men compared to CT-negative men for the following HPV outcomes: any HPV; oncogenic HPV; ≥1 HPV genotype; multiple HPV genotypes; HPV vaccine types; HPV 11, and HPV 18 ($P \leq 0.05$). HPV prevalence was higher in HSV-2 seropositive men than in HSV-2 seronegative men for the following HPV outcomes: any HPV; oncogenic HPV; non-oncogenic HPV only; ≥1 HPV genotype; and multiple HPV genotypes ($P \leq 0.05$).

Bivariable and multivariable analyses (approach 1) examining associations between the four HPV outcomes and the two main exposures (CT infection and HSV-2 serostatus) are presented in Table 3. In bivariable and multivariable analyses, CT infection was independently associated with any HPV (aOR 2.19, 95%CI: 1.13-4.24), oncogenic HPV (aOR 3.10, 95%CI: 1.53-6.28), and multiple HPV infections (aOR 3.43, 95%CI: 1.69-6.95). In the case of non-oncogenic HPV only, the association was marginally significant in the bivariable analysis (crude OR 2.16, 95%CI: 1.00-4.68, $P=0.050$) and was not significant in the multivariable analysis (aOR 1.95, 95%CI: 0.88-4.32, $P=0.101$). HSV-2 seropositivity was significantly associated with any HPV (aOR 1.25, 95%CI: 1.02-1.52), non-oncogenic HPV only (aOR 1.38, 95%CI: 1.08-1.75), and multiple HPV infections (aOR 1.33, 95%CI: 1.06-1.68). However, in the case of oncogenic HPV, HSV-2 serostatus was significantly associated in the bivariable analysis (crude OR 1.52, 95%CI: 1.24-1.85) and marginally significant in multivariable analyses (aOR 1.26, 95%CI: 1.00-1.59, $P=0.051$).

Multivariable logistic regression analyses were performed in two additional ways (see methods). In approach 2, addition of the covariates mentioned above did not improve the model based on the likelihood ratio test, and did not significantly alter the association between either CT infection and HSV-2 serostatus with the four HPV outcomes by >5%. Using approach 3, the association between multiple HPV infections and HSV-2 seropositivity was not significant (aOR 1.25; 95%CI 0.99-1.57; $P=0.056$); all other results were comparable.

Stratified analyses for the association between CT infection and the four HPV outcomes are presented in Table 4a. A significant association for all outcomes was found in the group that reported ≥2 RSP, except for the association between CT infection and non-oncogenic HPV only (aOR 3.22, 95%CI: 0.86 - 12.06). Stratified analyses by LSP for the association between HSV-2 serostatus and the four HPV outcomes are shown in Table 4b. HSV-2 serostatus was significantly associated with the four HPV outcomes among men reporting 0-5 LSP, but this association was weaker among men reporting 6-14 LSP, and absent among men reporting ≥15 LSP. The same trend was observed for the three other HPV outcomes.

In additional stratified analyses by country CT was positively associated with HPV in each country; however, the strength of these associations was highest in the US, followed by Mexico, and weakest in Brazil. HSV-2 was positively associated with HPV in each country (data not shown).

Discussion

This is one of the first studies investigating the association of genital HPV infection with CT infection and HSV-2 serostatus in men. After adjusting for confounding factors, CT

infection was positively associated with any HPV, oncogenic HPV, and multiple HPV infections; HSV-2 was positively associated with any HPV, non-oncogenic HPV, and multiple HPV infections. In expanded models adjusting for additional variables generally focused on sexual behavior, ORs for CT and HSV-2 were unchanged and remained significant. Thus, additional correction for sexual behavior did not alter our conclusion, suggesting that in this cross-sectional analysis confounding due to sexual behavior was well controlled.

We measured prevalent genital HPV infection, while HSV-2 seropositivity represented cumulative sexual exposure. The association between two markers reflecting different stages of infection may be questioned. However, as HSV-2 after a primary infection leads to clinical recurrences, HSV-2 seropositive individuals may have frequent reactivations of genital HSV-2 eruptions (54% of the men with genital HSV-2 infection or HSV-2 antibodies had 6 or more recurrences per year) (27).

The association we observed between CT infection and HPV infection may raise some questions as these infections affect different anatomical sites: CT infection is located in the urethra, whereas HPV infection is mostly located at the coronal sulcus, penile shaft, and scrotum. The biological mechanism responsible for an association between these two STIs therefore remains to be elucidated.

Men who reported a current penile discharge or dysuria and men who were being treated for an STI were excluded from participation in this study. Therefore we are only measuring associations between asymptomatic CT and HPV infections. Although this is a limitation, greater than 50% of CT infections in males are asymptomatic (28). This explains why, despite the exclusion criteria, we observed 1.6% of men had a CT infection.

A limitation of the current study is the inability to address temporality. It is unclear whether the STI was transmitted concurrently with HPV, or preceded or followed HPV. In the case of HSV-2, temporality may not be an issue as HSV-2 seroprevalence indicates past infections that may have influenced the current HPV infection. Although HSV-2 assessed with antibodies does not distinguish between oral, anal or genital HSV-2 infection, HSV-2 seropositivity indicates genital herpes in almost all patients (29). Since both CT and HPV infection are current infections, it is challenging to draw conclusions on temporality of these two infections. Another limitation is the relatively low prevalence of each HPV type, therefore it was not possible to investigate CT/HSV-2 associations with individual HPV types.

Our study concerns a well-characterized international cohort of men with a wide age range, a large sample size, with extensive data regarding demographic and sexual behavior characteristics, high rate of specimen adequacy (98%), and comprehensive laboratory ascertainment. As such, the study provides a good starting point to investigate the association of CT infection and HSV-2 serostatus with HPV in more detail.

Our results are in good agreement with previous studies in women (10;30). A prospective study among men from Denmark found CT infection to be a risk factor for acquiring an additional HPV type in HPV-positive men (17). A recent study investigating heterosexual couples in Rwanda found a moderate association between high-risk HPV infection and HSV-2 seropositivity (OR=2.4, 95%CI: 1.2-5.0) in men (19). A Japanese study in men found CT not to be associated with HPV in a bivariable analysis, although in a multivariable analysis STIs were significantly associated with HPV infection (OR=2.41, 95%CI: 1.04-5.61) (20). In a study among male attendees of the STI clinic in the Netherlands, an association between history of Chlamydia and gonorrhea with HPV infection was observed (18). Among Kenyan men, CT infection was significantly independently associated with

HPV presence in the glans (aOR=1.87, 95%CI 1.24-2.81) but not the shaft, and HSV-2 serostatus was only marginally independently associated with HPV presence in the glans (aOR=1.25, 95%CI 1.01-2.81) (21).

In stratified analyses regarding CT and HPV infection, the strength of the association increased with increasing number of RSP. Men with 2 RSP may be acquiring both STIs simultaneously from their high-risk partners. As the association among men with 2 RSP was stronger than in the whole study population this may indicate residual confounding due to sexual behavior. However, it is also possible that CT increases risk of HPV acquisition or persistence through a biological interaction. In an analogy of the hypothesized interaction between CT and HPV at the cervix, CT induces an inflammatory reaction that indirectly may increase HPV persistence through decreases in cell-mediated immunity (3). When stratifying by LSP, the strength of the HSV-2 and HPV association increased with decreasing number of LSP. In the case of HSV-2, the biological interaction in men reporting 6 LSP may be obscured by riskier sexual behavior that overwhelms the added risk conferred by HSV-2.

Further prospective studies in men are needed to clarify the role of STIs as a cofactor in HPV natural history, specifically HPV acquisition and persistence. There may be concern that STIs are surrogates for higher-risk behaviors that increase the exposure to HPV. Our stratified analyses for HSV-2 indicate the contrary. Prospective studies in men are needed in which multiple measures of HPV infections and STIs would help differentiate between STIs as true HPV cofactors or as indicators of sexual behavior. Overall, these results show that after correcting for sexual behavior, men infected with CT and men seropositive for HSV-2 are at higher risk for HPV infection.

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Baseline characteristics and sexual history of the study population (recruited July 2005-September 2009) by Chlamydia trachomatis infection and herpes simplex virus type 2 serostatus.

Table 1

	<i>Chlamydia trachomatis</i>			Herpes simplex virus type 2		
	Pos n	Total N	%	Pos n	Total N	%
Demographic characteristics						
Age in years	64	3,968	1.6%	811	3,965	20.4%
18-30	47	1,936	2.4%	197	1,935	10.2%
31-44	14	1,524	0.9%	424	1,523	27.8%
45-70	3	508	0.6%	190	507	37.5%
Country of residence						
USA	15	1,267	1.2%	163	1,265	12.9%
Brazil	32	1,387	2.3%	533	1,388	38.4%
Mexico	17	1,314	1.3%	115	1,312	8.8%
Marital status						
Single or never married	34	1,782	1.9%	279	1,781	15.7%
Married	14	1,358	1.0%	296	1,358	21.8%
Cohabiting	8	477	1.7%	111	477	23.3%
Divorced, separated, or widowed	8	341	2.4%	125	339	36.9%
Refused to answer	0	10	0%	0	10	0%
Education in Years						
12 years completed	35	1,949	1.8%	456	1,947	23.4%
>12 years completed	29	2,006	1.5%	352	2,005	17.6%
Refused to answer	0	13	0%	3	13	23.1%
Health behavior						
Smoking status						
Never	38	2,288	1.7%	449	2,283	19.7%
Former	7	729	1.0%	174	730	23.8%
Current	19	931	2.0%	185	932	19.9%
Refused to answer	0	20	0%	3	20	15.0%
Circumcision status						

	<i>Chlamydia trachomatis</i>				Herpes simplex virus type 2			
	Pos n	Total N	%	<i>p</i> ^a	Pos n	Total N	%	<i>p</i> ^a
No	43	2,545	1.7%	0.608	592	2,544	23.3%	<0.001
Yes	21	1,423	1.5%		219	1,421	15.4%	
Sexual behavior								
Lifetime number of female sexual partners								
0	2	156	1.3%	0.175	40	156	25.6%	<0.001
1-19	24	1,909	1.3%		264	1,908	13.8%	
20	33	1,457	2.3%		401	1,455	27.6%	
Never sex	3	226	1.3%		40	225	17.8%	
Refused to answer	2	220	0.9%		66	221	29.9%	
Recent female sexual partners in past 3-6 months								
0	11	967	1.1%	0.026	219	969	22.6%	0.055
1-2	32	2,121	1.5%		402	2,119	19.0%	
3	17	524	3.2%		121	522	23.2%	
Never sex	3	226	1.3%		40	225	17.8%	
Refused to answer	1	130	0.8%		29	130	22.3%	
Lifetime number of male anal sexual partners								
0	51	3,153	1.6%	0.364	566	3,151	18.0%	<0.001
1-2	2	235	0.9%		65	235	27.7%	
3	8	294	2.7%		116	295	39.3%	
Never sex	3	226	1.3%		40	225	17.8%	
Refused to answer	0	60	0%		24	59	40.7%	
Recent male anal sexual partners in past 3 months								
0	55	3,477	1.6%	0.518	659	3,475	19.0%	<0.001
1	6	240	2.5%		100	240	41.7%	
Never sex	3	226	1.3%		40	225	17.8%	
Refused to answer	0	25	0%		12	25	48.0%	
Age of sexual debut in years								
15	26	1,105	2.4%	0.143	313	1,106	28.3%	<0.001
>15	32	2,441	1.3%		409	2,438	16.8%	
No vaginal partners	2	151	1.3%		39	151	25.8%	

	<i>Chlamydia trachomatis</i>				Herpes simplex virus type 2			
	Pos n	Total N	%	<i>p</i> ^a	Pos n	Total N	%	<i>p</i> ^a
Never sex	3	226	1.3%		40	225	17.8%	
Refused to answer	1	45	2.2%		10	45	22.2%	
Ever diagnosed with an STI								
No	51	3,202	1.6%	0.955	549	3,199	17.2%	<0.001
Yes	10	640	1.6%		232	640	36.3%	
Refused to answer	3	126	2.4%		30	126	23.8%	
Condom use during vaginal or anal sexual past 3-6 months								
Always condom	13	738	1.8%	0.323	148	737	20.1%	0.301
half the time a condom	15	726	2.1%		143	727	19.7%	
< half the time a condom	11	450	2.4%		99	449	22.1%	
Never a condom	20	1,486	1.4%		324	1,484	21.8%	
No vaginal/anal sex	2	327	0.6%		56	328	17.1%	
Never sex	3	226	1.3%		40	225	17.8%	
Refused to answer	0	15	0%		1	15	6.7%	
Partner diagnosed with genital warts during the past 6 months								
No	35	2,578	1.4%	0.028	500	2,576	19.4%	0.049
Yes	1	210	0.5%		52	210	24.8%	
Don't know	28	1,174	2.4%		259	1,173	22.1%	
Refused to answer	0	6	0%		0	6	0%	
Partner diagnosed with an STI during past 6 months								
No	26	1,919	1.4%	0.149	338	1,915	17.7%	<0.001
Yes	8	641	1.3%		194	642	30.2%	
Don't know	30	1,400	2.1%		278	1,400	19.9%	
Refused to answer	0	8	0.0%		1	8	12.5%	

^a *p* based on the Chi-squared test: Responses that were refusals were not included for calculations of *p*-values except for refusals of the 'lifetime number of female sexual partners' and 'recent female sexual partners in past 3-6 months' characteristics

Abbreviations: STI: Sexually transmitted infection, Pap test: Papanicolaou test, HPV: Human papillomavirus, Pos: Positive

Data are missing for: *Chlamydia trachomatis* infection status (n = 3), Herpes simplex virus type 2 serostatus (n = 6)

Table 2
Prevalence of human papillomavirus infection in the HIM study population by Chlamydia trachomatis infection and herpes simplex virus type 2 serostatus

	<i>Chlamydia trachomatis</i>				Herpes simplex virus type 2 serostatus			
	Positive (n=64)		Negative (n=3,904)		Positive (n=811)		Negative (n=3,154)	
	n	%	n	%	n	%	n	%
Positive for any HPV	53	82.8%	2,594	66.4%	599	73.9%	2,045	64.8%
Positive for oncogenic HPV	33	51.6%	1,148	29.4%	265	32.7%	914	29.0%
Positive for non-oncogenic HPV only	16	25.0%	881	22.6%	233	28.7%	664	21.1%
Positive for unclassified HPV ^b	4	6.3%	565	14.5%	101	12.5%	467	14.8%
Positive for 1 HPV genotype	49	76.6%	2,029	52.0%	498	61.4%	1,578	50.0%
Positive for multiple HPV genotypes	36	56.3%	1,109	28.4%	281	34.7%	861	27.3%
Positive for HPV 6, 11, 16 or 18	19	26.7%	610	15.6%	125	15.9%	502	15.4%
Positive for HPV 6	7	10.9%	233	6.0%	41	5.1%	199	6.3%
Positive for HPV 11	3	4.7%	51	1.3%	11	1.4%	43	1.4%
Positive for HPV 16	9	14.1%	298	7.6%	64	7.9%	242	7.7%
Positive for HPV 18	4	6.3%	87	2.2%	18	2.2%	72	2.3%
Negative for HPV ^c	11	17.2%	1,310	33.6%	212	26.1%	1,109	35.2%

^a *P* values based on Pearson Chi-squared test:

^b Specimens that tested positive for HPV PCR but negative for HPV genotyping were considered HPV unclassified.

^c Specimens negative for HPV on both PCR and genotyping

Abbreviations: HPV: Human papillomavirus

Table 3
Association between HPV infection and *Chlamydia trachomatis* infection and, HPV infection and herpes simplex virus type 2 serostatus: bivariable and multivariable analyses

Outcome	Exposure	Bivariable OR (95% CI)	Multivariable aOR (95% CI)
Positive for Any HPV	CT neg	1	1
	CT pos	2.43 (1.27 - 4.67)	2.19 (1.13 - 4.24) ^a
Positive for Oncogenic HPV	CT neg	1	1
	CT pos	3.42 (1.72 - 6.80)	3.10 (1.53 - 6.28) ^a
Positive for Non-Oncogenic HPV only	CT neg	1	1
	CT pos	2.16 (1.00 - 4.68)	1.95 (0.88 - 4.32) ^a
Multiple HPV (positive for >1 HPV)	CT neg	1	1
	CT pos	3.87 (1.96 - 7.63)	3.43 (1.69 - 6.95) ^a
Positive for Any HPV	HSV-2 neg	1	1
	HSV-2 pos	1.53 (1.29 - 1.82)	1.25 (1.02 - 1.52) ^b
Positive for Oncogenic HPV	HSV-2 neg	1	1
	HSV-2 pos	1.52 (1.24 - 1.85)	1.26 (1.00 - 1.59) ^b
Positive for Non-Oncogenic HPV only	HSV-2 neg	1	1
	HSV-2 pos	1.84 (1.49 - 2.26)	1.38 (1.08 - 1.75) ^b
Multiple HPV (positive for >1 HPV)	HSV-2 neg	1	1
	HSV-2 pos	1.71 (1.40 - 2.08)	1.33 (1.06 - 1.68) ^b

^a **Multivariable models are adjusted for:** age (categorical), country of residence, education, smoking status (ever), recent female sexual partners in past 3-6 months and recent male anal sexual partners in past 3 months

^b **Multivariable models are adjusted for:** age (categorical), country of residence, education, smoking status (ever), lifetime number of female sexual partners and lifetime number of male anal sexual partners

Abbreviations: OR: Odds ratio, aOR: adjusted Odds ratio, HPV: Human papillomavirus, CI: Confidence interval, CT: *Chlamydia trachomatis*

Table 4
a: Association between HPV infection and CT infection stratified by recent number sexual partners during the pas 3-6 months

Outcome	Exposure	Recent number of sexual partners ^b				
		0 n = 723	1 n = 1,608	2 n = 1,107		
		Bivariable OR (95% CI)	Bivariable OR (95% CI)	Bivariable OR (95% CI)	Multivariable ^a aOR (95% CI)	Multivariable ^a aOR (95% CI)
Positive for Any HPV	CT neg	1	1	1	1	1
	CT pos	1.44 (0.26 - 7.90)	1.59 (0.28 - 8.93)	1.21 (0.42 - 3.45)	1.26 (0.44 - 3.63)	3.35 (1.01 - 11.10)
Positive for Oncogenic HPV	CT neg	1	1	1	1	1
	CT pos	2.88 (0.48 - 17.39)	3.49 (0.55 - 22.06)	1.47 (0.45 - 4.86)	1.59 (0.47 - 5.32)	4.22 (1.24 - 14.36)
Positive for Non-Oncogenic HPV only	CT neg	1	1	1	1	1
	CT pos	1.06 (0.10 - 11.79)	0.85 (0.08 - 9.67)	1.24 (0.33 - 4.65)	1.21 (0.31 - 4.70)	3.22 (0.86 - 12.06)
Multiple HPV (pos. for >1 HPV)	CT neg	1	1	1	1	1
	CT pos	3.16 (0.52 - 19.11)	3.13 (0.49 - 20.20)	1.62 (0.49 - 5.36)	1.86 (0.55 - 6.29)	4.23 (1.25 - 14.37)

b: Association between HPV infection and herpes simplex virus type 2 serostatus stratified by lifetime number sexual partners

Outcome	Exposure	Lifetime number of sexual partners ^b				
		0-5 n = 1,320	6-14 n = 1,015	15 n = 1,149		
		Bivariable OR (95% CI)	Bivariable OR (95% CI)	Bivariable OR (95% CI)	Multivariable ^a aOR (95% CI)	Multivariable ^a aOR (95% CI)
Positive for Any HPV	HSV-2 neg	1	1	1	1	1
	HSV-2 pos	2.00 (1.37 - 2.91)	1.43 (0.99 - 2.06)	0.82 (0.62 - 1.11)	1.45 (0.97 - 2.19)	0.91 (0.67 - 1.24)
Positive for Oncogenic HPV	HSV-2 neg	1	1	1	1	1
	HSV-2 pos	1.59 (1.05 - 2.40)	1.43 (0.99 - 2.06)	0.82 (0.62 - 1.11)	1.45 (0.97 - 2.19)	0.91 (0.67 - 1.24)

b: Association between HPV infection and herpes simplex virus type 2 serostatus stratified by lifetime number sexual partners

Outcome	Exposure	Lifetime number of sexual partners ^b				
		0-5 n = 1,320	6-14 n = 1,015	15 n = 1,149		
		Bivariable OR (95% CI)	Multivariable ^a aOR (95% CI)	Bivariable OR (95% CI)	Multivariable ^a aOR (95% CI)	Bivariable OR (95% CI)
Positive for Non-Oncogenic HPV only	HSV-2 pos	2.18 (1.36 - 3.52)	2.14 (1.27 - 3.61)	1.26 (0.83 - 1.91)	1.38 (0.86 - 2.21)	0.75 (0.54 - 1.04)
	HSV-2 neg	1	1	1	1	1
	HSV-2 pos	2.37 (1.48 - 3.82)	1.81 (1.07 - 3.08)	1.72 (1.12 - 2.64)	1.63 (1.01 - 2.63)	0.92 (0.65 - 1.31)
Multiple HPV (pos. for >1 HPV)	HSV-2 neg	1	1	1	1	1
	HSV-2 pos	2.21 (1.34 - 3.65)	1.80 (1.04 - 3.14)	1.56 (1.04 - 2.35)	1.53 (0.98 - 2.41)	0.79 (0.57 - 1.09)
						0.92 (0.65 - 1.29)

Abbreviations: OR: Odds ratio, aOR: adjusted Odds ratio, pos.: positive, HPV: Human papillomavirus, CI: Confidence interval, CT: Chlamydia trachomatis, HSV-2: Herpes simplex virus type 2

^a All multivariable models are adjusted for: age (categorical), country of residence, education and smoking status (ever)

^b Number of sexual partners represents both male and female partners.