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Serum Antibodies to Human Papillomavirus Type 6, 11, 16 and 18 and Their Role in the Natural History of HPV Infection in Men

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Serum Antibodies to Human Papillomavirus (HPV) Type 6, 11, 16 and 18

and Their Role in the Natural History of HPV Infection in Men

by

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of the requirements for the degree of
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ABSTRACT

Our understanding of humoral immune response to human papillomavirus (HPV) infection has been mainly derived from studies in women. Very little is known about humoral immune response to HPV in men. There is also a growing interest in understanding the burden of HPV exposure in the subgroups of the male population, including men who have sex with women (MSW), men who have sex with men (MSM) and men who have sex with both men and women (MSMW). This dissertation was undertaken to understand and characterize humoral immune response, measured by detectable serum antibody IgG, to HPV 6, 11, 16 and 18 infection, to estimates seroprevalence of HPV 6, 11, 16 and 18, to determine the associations of socio-demographic and sexual behavioral factors with seroprevalence of individual HPV types, and to evaluate the role of serum antibodies in the subsequent acquisition of infection with the same HPV type, genetically related and un-related HPV types.

Three studies that compose of this dissertation were conducted within the framework of two longitudinal studies of HPV infection in men: a single-site natural history study of male residents of Tucson, Arizona (the 1st study: N=285); and a multi-national natural history study of healthy men residing in São Paulo, Brazil, Cuernavaca, Mexico, and Tampa, Florida (the 2nd study: N=1477; the 3rd study: N=2187). Men were recruited using similar eligibility criteria in both natural history studies and followed every 6 months for a maximum of 18 months in the single-site study and 48 months in the multi-national study. HPV DNA status was assessed using the PGMY09/11 L1 consensus primer system and the Linear Array HPV Genotyping Protocol. Testing of

serum antibodies to HPV 6, 11, 16 and 18 was performed with virus-like particle-based ELISA assays.

Data from our studies indicate that exposure to HPV 6, 11, 16 and 18, the four HPV types targeted in the currently license HPV vaccines, is common. Of 285 male residents of Tucson, Arizona, 28.8% of them were seropositive to HPV 16 and/or 18 at study entry. Similarly, approximately one third of 1477 participants of the multi-national male HPV natural history study were seropositive to at least one vaccine HPV type, with the percentage of 21.8% in U.S. site, 33.4% in Mexico site, and 49.1% in Brazil site. It is also noted that seroprevalence of individual vaccine HPV types is greatly elevated among men of different sexual practices. Seroprevalence of HPV 6, 11, 16 and/or 18 was twice as high among MSM and MSMW compared to MSW. Likewise, seroprevalence of individual HPV types was two fold or higher among MSW and MSMW.

Our findings suggest that the predominant predictors of seropositivity to HPV 6, 11, 16 and 18 are age and same-sex sexual behaviors. Seroprevalence increased with age among young-to-middle-aged men with significant upward age trends observed for HPV 11, 16 and 18. MSM, compared to MSW, more likely to be seropositive to HPV 16 or 18. Similarly, men who practiced same-sex anal sex, compared to those who did not, were significantly more likely to be seropositive to HPV 6, 11, 16 and 18, respectively.

Among 276 men free of HPV 16 at enrollment in Tucson, We did not detect statistically significant associations between the baseline serum antibodies to HPV 16 and/or 18 and subsequent risk of infection with homogeneous HPV types or related-HPV types. Of 2187 men residing in three countries who tested HPV 16 negative at enrollment, the risk of subsequent HPV 16 infection was not associated with enrollment HPV 16 serum antibodies status.

Our data provide important estimates of population exposure to vaccine HPV types for future studies modeling potential vaccine impact and vaccine cost effectiveness

in men. Our findings also support strategic vaccination of males as an effective preventive measure for HPV-related diseases and cancers in men and their sex partners, men and women alike.

CHAPTER 1:

INTRODUCTION

Human papillomavirus (HPV) infection is one of the most common sexually transmitted infections (STIs) in the United States [1]. HPV infection in men contributes significantly to infection and subsequent cervical disease in women [2-5]. Studies of HPV infection in heterosexual couples have shown that males' sexual behavior and HPV infection status are significantly associated with their female partners' risk of precancerous lesion and cervical cancer [2-8]. High prevalence and high incidence of HPV infection in men are also associated with lesions [9-11] and cancers of anal canal and penis [12-16]. It has been reported in recent studies [17-19] that 25.8-72.9% of HIV-negative adult men test positive for genital HPV. Anal and oral HPV, though less common, is present in 8.0-16.6% [20-22], and 2.9-7.6% [23] of HIV-negative adult men, respectively.

There is also a growing interest in understanding the burden of HPV infection among men with different sexual practices including men who have sex with women (MSW), men who have sex with men (MSM) and men who have sex with both men and women (MSMW). Compared to MSW, MSM and MSMW are several times more likely to be infected with HPV at different anatomic sites, and hence may be at increased risk for HPV-associated diseases and cancers [24-28].

HPV serology reflects cumulative exposures to type-specific HPV over time and across anatomic sites. It provides a useful means for estimating cumulative HPV exposures in a population and can provide insights into the natural history of HPV

infection. With the recent licensure of the quadrivalent HPV vaccine for use in males, information on seroprevalence of vaccine-type HPV (HPV 6, 11, 16 and 18) in the general male population is needed to provide guidance for strategic planning of vaccination.

Our understanding of humoral immune response to HPV infection has been mainly derived from serological studies in women with a focus on serum antibodies to HPV 16. In contrast, little is known about humoral immune response to HPV in men. This dissertation was undertaken to understand and characterize humoral immune response, measured by detectable serum antibody IgG, to HPV 6, 11, 16 and 18 infection, to determine the associations of socio-demographic and sexual behavioral factors with seroprevalence of individual HPV types, and to evaluate the role of serum antibodies in the prevention of subsequent infection with the same HPV type, genetically related and un-related HPV types. Three studies were conducted to achieve these objectives. The first is a study of serum antibodies to HPV 16 and/or 18, its relationship with potential risk factors and subsequent development of HPV 16 and/or 18 infections among a cohort of U.S. men; in the second study, investigation of serum antibodies and associated risk factors was expanded to four HPV types targeted in current HPV vaccines (HPV 6, 11, 16 and 18) among a large multi-national male cohort; and the third study further examined if risk of acquiring HPV 16 infection over a three-year follow-up period is modified by the enrollment HPV 16 serum antibody status in the same cohort of men.

The sources of study population are two natural history studies of HPV infection in men: (1) a single-site, prospective study of male HPV infection conducted between September 2003 and December 2005 and funded by the Arizona Disease Control Research Commission, consisting of male residents aged 18-44 from Tucson, Arizona; (2) an ongoing multi-national prospective study of HPV Infection in Men (*HIM* Study)

beginning in June 2005, comprising men aged 18-70 residing in Tampa, United States, São Paulo, Brazil, and Cuernavaca, Mexico.

CHAPTER 2:

THE FIRST MANUSCRIPT

EPIDEMIOLOGIC FACTORS ASSOCIATED WITH SEROPOSITIVITY TO HUMAN PAPILLOMAVIRUS TYPE 16 AND 18 VIRUS-LIKE PARTICLES (VLPS) AND RISK OF SUBSEQUENT INFECTION IN MEN

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ABSTRACT

Our understanding of humoral response to HPV infection has been mainly derived from studies in women. The role of serum antibodies in the natural history of HPV in men has yet to be investigated. Data from 285 male participants of a natural history study were used to determine epidemiologic factors associated with HPV 16/18 seropositivity and explore the role of HPV 16 and 18 serum antibodies in subsequent HPV infections**.** Serum antibodies were detected by use of HPV 16 and 18 virus-like particles ELISA. Logistic regression and generalized estimating equation was employed for evaluation of risk factors. The risk of subsequent HPV infection by baseline antibody status was assessed by Incidence Rate Ratio and its confidence intervals. Men aged 36-44 compared to men aged 18-25 were four times more likely to be seropositive to HPV 16/18. In addition, being divorced, separated or widowed, being former smoker and having sex with men was positively and independently associated with HPV 16/18 seropositivity. Our findings on the protective role of HPV 16 or 18 serum antibodies in subsequent infection were inconclusive. Large prospective studies are warranted to adequately address questions on the role of natural immunity in the natural history of HPV infections in men.

INTRODUCTION

Type-specific Human Papillomavirus (HPV) serology can provide insights into the natural history of HPV infection and associated HPV diseases. Our understanding of HPV serology has been mainly derived from studies in women with a focus on serum antibodies to HPV 16. A limited number of studies have evaluated the prevalence of HPV 16 serum antibodies and its determinants, and fewer have evaluated the prevalence of serum antibodies to other HPV types. Among 21 published studies that investigated serum antibodies to HPV 16 L1 VLPs and factors associated with HPV 16 seropositivity, only nine enrolled men [29-37]. Only two published studies to date have examined the potential protective role of serum antibodies in subsequent infection in women [38, 39] and none have been conducted among men. To address this information gap, we investigated epidemiologic factors associated with HPV 16/18 seropositivity and explored the role of HPV 16 and 18 serum antibodies in subsequent HPV infections among 285 U.S. men.

METHODS

Study Population. A prospective cohort was established for the HPV Infection in Men study in Tucson, Arizona between September 2003 and December 2005. Details of this cohort have been reported previously [18, 40]. In brief, 337 men (89.4% of eligible men screened) aged 18-44 years were enrolled. Men were residents of southern Arizona who reported no prior diagnosis of penile or anal cancers, or genital warts; and no current diagnosis or treatment of sexually transmitted infections (STIs) including genital warts, genital Herpes, Chlamydia, gonorrhea, syphilis, non-gonococcal urethritis, hepatitis B virus, hepatitis C virus and Human Immunodeficiency Virus (HIV) infection. All participants were consented prior to enrollment. Men were followed at 6-month intervals for approximately 18 months. At each study visit, participants completed a self-administered risk factor questionnaire, and had penile and scrotal cell samples and a

venous blood sample collected. The current analysis included 285 men who completed at least 2 study visits, had adequate samples for HPV DNA detection and serum antibody testing, and had available questionnaire information from each visit.

HPV DNA Testing. Cellular materials collected at each visit were tested for presence of HPV DNA using the PGMY09/11 L1 consensus primer system. HPV genotyping was conducted using the reverse line blot method [41] to detect 37 genital HPV types, regardless of the PCR result (Roche Molecular Diagnostics). Only samples that tested β-globin positive were deemed adequate and included in this analysis.

HPV Serum Antibody Testing. HPV VLPs were prepared using BSC-1 cells (monkey kidney cell line) infected with the recombinant vaccinia virus expressing the L1 gene of HPV 16 and 18. IgG antibodies to HPV 16 and 18 VLPs were measured with enzyme-linked immunoassay (ELISA) as described previously [42]. Monoclonal antibodies used for Capture ELISA were H16.V5-HPV-16 and H18.J4-HPV-18 kindly provided by Dr. N. Christensen at Pennsylvania State Medical Center, Hershey, PA. The cutpoints were determined using a serum bank from children less than 10 years old with no prior history of warts, and calculated as the average reactivity plus two standard deviations [42].

Statistical Analysis. To determine factors associated with HPV 16/18 serum seropositivity, we employed logistic regression models with HPV 16/18 serum antibody status as the dependent variable. All participants with available serum antibody measurements were included in the analysis regardless of their baseline serum antibody status. Repeated measurements taken throughout the study period were incorporated to capture changes in serum antibody status. The Generalized Estimating Equation (GEE) [43] was applied to account for the correlation between repeated observations of the same individuals. An unstructured working correlation was assumed. We examined the likelihood of seropositivity in relation to sociodemographic characteristics, such as age,

race, marital status, and education; and lifestyle and behavioral factors obtained at enrollment, including cigarette smoking, average alcohol use, circumcision, age at first sexual intercourse, sexual practice, and lifetime number of sex partners. We also included risk factors that changed over time and were repeatedly surveyed at each study visit, including the number of recent sex partners, self-reported STI status for self and partner since last visit, recent condom use and HPV 16/18 DNA status. Factors demonstrating a p-value of 0.10 or smaller in univariable models were included in the multivariable models and further evaluated by score chi-squared statistics. Odds Ratio (OR) and 95% confidence intervals (CIs) were calculated. The risk of subsequent infection with type-specific or group-specific HPV was evaluated among men who were DNA negative to corresponding HPV type(s) at study entry, and measured by Incidence Rate Ratio (IRR) and its 95% CIs calculated using the method proposed by Rothman and Greenland [44].

RESULTS

Two hundred and eighty-five men were followed for approximately 18 months. Of the 285 men, 153 (53.7%) completed four study visits including the enrollment visit, 87 (30.5%) completed three visits, and 45 (15.8%) only completed two visits. The median duration of follow-up was 15.5 months (range, 3.7–24.7 months) and the median follow-up interval was 5.3 months. The mean age of the cohort was 29.8 years (SD: 8.1). Overall, HPV 16/18 seroprevalence was 28.8% at study entry, and 26.4, 30.0 and 29.8% at the 6-, 12- and 18-month visit, respectively. At enrollment, 14.8% of men were seropositive to HPV 16, followed by 14.4, 18.9 and 24.8% of men at each follow-up visit. The seroprevalence for HPV 18 was 21.1, 18.8, 21.6 and 19.9% throughout the study period.

In crude analyses, HPV 16/18 serum antibody status was significantly associated with age, marital status, smoking status, sexual practice and the number of lifetime

sexual partners (Table 2.1). Characteristics that remained statistically significantly associated with serum antibody status in the final adjusted model included age, marital status, cigarette smoking and sexual practice. Compared to men aged 18-25 years, men aged 36-44 years were more likely to be seropositive to HPV 16/18 (OR, 4.2, 95% CI: 2.2-8.1). Divorced, separated or widowed men compared to single men were twice as likely to be seropositive to HPV 16/18 (OR, 2.3, 95% CI: 1.02-5.3). Smokers were more likely to be seropositive compared with never smokers (former smoker: OR, 2.0, 95% CI: 1.03-3.9; current smoker: OR, 1.5, 95% CI: 0.8-2.8), although the latter did not reach statistical significance. A higher likelihood of seropositivity for men having sex with men (MSM) compared to men having sex with women only (MSW) was observed (OR, 2.6, 95% CI: 1.05-6.7).

We examined the potential protection that HPV 16 and 18 serum antibodies confer against subsequent infection by assessing the risk of infection with homologous HPV types over the study period among men who had no detectable HPV DNA of interest at the baseline by their baseline serum antibody status (Table 2.2). The risk of subsequent infection with HPV 16 among 276 men who had no detectable HPV 16 infection at study entry did not differ significantly by their baseline HPV 16 serum antibody status (IRR, 1.1, 95% CI: 0.3-4.0). Similarly, no statistically significant difference in the risk of subsequent infection with HPV 18 was detected between HPV 18 seropositive and seronegative men at the baseline (IRR, 1.8, 95% CI: 0.2-20.2).

We also explored the potential cross-protection provided by HPV 16 or 18 antibodies (Table 2.2). Seropositivity to HPV 16 appeared to be protective against new infections with HPV 16-related types (HPV 31, 33, 35, 52, 58 and 67) with an IRR of 0.6 (95% CI: 0.1-2.6), although the IRR did not achieve statistical significance. Similarly, seropositivity to HPV 18 yielded a statistically insignificant IRR of 0.6 (95% CI: 0.2-2.0) for infection with HPV 18-related types (HPV 39, 45, 59, 68, and 70). No significant

reduction in the risk of subsequent infection with other HPV types was observed for HPV 16 or 18 seropositive men as compared with seronegative men.

DISCUSSION

This is one of few studies to examine factors associated with HPV serum antibody status in men and the first study to explore the protective role of serum antibodies against future infections in men. In this study the predominant predictor of HPV 16/18 seropositivity was age. Men aged 36-44 years were four times more likely to be seropositive than men aged 18-25 years. In previous studies, age has been positively associated with seroprevalence in both men and women [33, 35, 45-48]. In two studies that included participants across a broad age range an inverse U-shaped association with age was reported, with seroprevalence peaking at age 35-49 and declining with increasing age afterwards [45, 46]. The observed age effect in the current study is likely to be a result of cumulative lifetime sexual exposure to HPV infection.

In the current study, smoking status was significantly associated with HPV antibody status in men. Smokers, compared to never smokers, were more likely to be HPV 16/18 seropositive. Recent studies of HPV seroprevalence and smoking in men have yielded mixed results [31, 34, 48]. No significant association with smoking status or tobacco use was found in the studies of Kreimer et al. [31] or Stone et al. [34], while a significant association with current smoking was reported by Dunne et al. among male residents of two U.S. cities (OR, 1.9, 95% CI: 1.1-3.2) [48]. Our finding is in agreement with the suggested immune suppressive effect of tobacco smoking which may facilitate persistence of viral infection and result in a higher likelihood of seroconversion for smokers [49].

An important observation from the current study was that MSM and MSMW (men who had sex with both men and women) were more likely to be seropositive. Similar findings have been reported in other studies. Stone et al. demonstrated that MSM were

6 times more likely to be seropositive to HPV 16 [34]. Kreimer et al. reported a significant association (OR, 2.9, 95% CI: 1.2-7.1) between seropositivity to HPV 16/18/33 VLPs and same-sex oral sexual intercourse among male participants of an oral HPV study [31]. History of having same-sex anal or oral sexual intercourse may serve as a surrogate marker for increased sexual exposure to the virus via multiple transmission routes including oral-penile, oral-anal and penile-anal transmission. With the absence of information on oral and anal HPV infection among this cohort of men, we could only hypothesize that an increased risk of oral or anal HPV infection in addition to genital infection may have contributed to increased seroconversion observed among MSM and MSMW. This hypothesis is also supported by findings from several recent studies. D'Souza et al. reported a higher prevalence of oral HPV infection with genital HPV types among MSM and MSMW compared to heterosexual men [26]. A high prevalence of anal HPV infection was reported among HIV-positive MSM from HIV/AIDS clinics in Montreal [50] as well as HIV-negative MSM in a community-based study [51]. Further studies of serum antibody development following incident HPV infection at different anatomic sites are needed to test this hypothesis.

Results of the current study were inconclusive regarding whether HPV 16 or 18 serum antibodies were effective in protecting against subsequent infection with the homologous or phylogenetically related HPV types. A protective role of serum antibodies to HPV 16 was shown by Ho and colleagues in a prospective study of 247 female college students [38]. Women with a high titer level of IgG antibodies (OD>400-800) to HPV 16 in 2 or more consecutive visits had a lower risk for subsequent infection with HPV 16 (RR=0.49; p=0.037) and with HPV 16-related types (p=0.010) [38]. In contrast, no protective effect was reported by Viscidi et al. in the natural history study of 7,046 Guanacaste women for whom the risk of subsequent infection was measured at the follow-up visit scheduled 5-7 years after the baseline serology. It is likely that the

inconclusive results on protective effect of serum antibodies in this study compared with female studies are in part attributable to potential gender difference in viral shedding and antibody response as observed in several serology studies that enroll both men and women [31, 33-37], and further compounded by the relative small sample size in the this study. Therefore, caution should be taken in interpreting the inconclusive finding of this study in men.

The current study has a number of limitations. First, we only enrolled men aged 18-44 years. The narrow age range prohibits an evaluation of age effect in older men. Another limitation of the current study is that we were not able to evaluate the risk of subsequent infection based on a quantitative measure of antibody titer. The percent of HPV-infected men who seroconvert remains unknown, as is the longevity of serum antibodies in men. Serum antibodies elicited by distant, past infection may wane over time like serum antibodies to other viral infections such as hepatitis B virus, leading to potential exposure misclassification. Additional limitations include the relatively small sample size and limited duration of follow-up which may have lowered statistical power needed to detect a statistically significant difference in the risk of type-specific or group-specific infections given the low incidence for HPV types of interest.

In summary, the current study identified age, marital status, smoking status and sexual practice as factors significantly and independently associated with HPV 16/18 serum antibody status. We did not detect statistically significant associations between the baseline serum antibody status and subsequent risk of infection. Large prospective studies that employ quantitative assessment of HPV antibody titers are needed to adequately address fundamental questions regarding the role of natural immunity in the natural history of HPV infections in men.

Table 2.1 Factors associated with HPV-16/18 seropositivity in 285 men in Tucson, Arizona

Characteristics	Baseline Seroprevalence	Crude		Adjusted [§]	
	No. Subjects (% Seropositive)	OR	95% CI	OR	95% CI
Age					
18-25	113 (13.3)	1.0		1.0	
26-35	86 (19.8)	1.3	(0.7-2.4)	0.6	(0.3-1.2)
36-44	86 (58.1)	5.6	(3.4-9.3) *	4.2	(2.2-8.1) *
Race					
White	237 (29.1)	1.0		--	
Non-white	38 (26.3)	0.7	(0.3-1.3)	--	
Marital Status					
Single/Never married	182 (23.6)	1.0		1.0	
Married/Cohabiting	69 (34.8)	1.6	(0.99-2.7)	1.5	(0.8-2.6)
Divorced/Separated/Widow	34 (44.1)	3.2	(1.7-6.1) *	2.3	(1.02-5.3) *
Education					
High school graduate or less	69 (26.1)	1.0		--	
Some college/vocational school	98 (24.5)	1.0	(0.6-1.8)	--	
College graduate/graduate school	118 (33.9)	1.2	(0.7-2.1)	--	
Cigarette smoking					
Never	95 (21.1)	1.0		1.0	
Former	54 (33.3)	2.2	(1.2-4.1) *	2.0	(1.03-3.9) *
Current	67 (35.8)	1.8	(0.99-3.2)	1.5	(0.8-2.8)
Alcohol use (drinks per month)					
0-13	66 (33.3)	1.0		--	
14-52	103 (29.1)	1.0	(0.7-1.3)	--	
≥ 53	56 (21.4)	0.8	(0.5-1.1)	--	
Circumcision (clinical assessment)					
No	35 (31.4)	1.0		--	

Characteristics	Baseline Seroprevalence	Crude		Adjusted [§]	
	No. Subjects (% Seropositive)	OR	95% CI	OR	95% CI
Yes	250 (28.4)	1.1	(0.6-2.0)	--	
Age at first sexual intercourse					
<18	160 (33.1)	1.4	(0.9-2.2)	--	
≥18	113 (23.9)	1.0		--	
Sexual practice					
Sexual intercourse with women	242 (26.4)	1.0		1.0	
Sexual intercourse with men	13 (46.2)	2.4	(0.9-6.2)	2.6	(1.05-6.7) *
Sexual intercourse with both	13 (53.8)	2.9	(1.01-8.4) *	2.1	(0.7-5.8)
Lifetime no. of sex partners (either sex)					
0-4	82 (19.5)	1.0		1.0	
5-16	130 (23.1)	1.1	(0.6-1.8)	0.6	(0.3-1.3)
≥17	68 (51.5)	2.3	(1.3-4.1) *	1.0	(0.4-2.1)
No. of new sex partners in the past 3 months					
None	160 (27.5)	1.0		--	
One or more	94 (29.8)	1.0	(0.7-1.3)	--	
Diagnosed with other STI since last visit **					
No	204 (26.5)	1.0		--	
Yes	75 (36.0)	1.5	(0.9-2.4)	--	
Had partner(s) with other STI since last visit **					
No	103 (18.4)	1.0		--	
Yes	124 (34.7)	1.1	(0.9-1.5)	--	
Condom use in the past 3 months					
Never	86 (30.2)	1.0		--	
Sometimes	41 (34.1)	1.0	(0.7-1.5)	--	
Frequently	40 (25.0)	0.8	(0.5-1.1)	--	
Always	45 (20.0)	1.1	(0.7-1.8)	--	
HPV 16/18 infection(s)					

Characteristics	Baseline Seroprevalence	Crude		Adjusted §	
	No. Subjects (% Seropositive)	OR	95% CI	OR	95% CI
No	270 (29.3)	1.0		--	
Yes	9 (22.2)	1.2	(0.6-2.4)	--	

Note. OR: Odds Ratio. 95% CI: 95 percent confidence interval. * denotes statistical significance (α= 0.05)
§ The final model included age, marital status, cigarette smoking, lifetime number of sexual partners and sexual practice.
** Other STIs include genital warts, genital Herpes, Chlamydia, gonorrhea, syphilis, non-gonococcal urethritis, hepatitis B, hepatitis C and HIV.

Table 2.2 Risk of subsequent infection with type-specific and group-specific HPV by baseline serum antibody status.

Baseline Serum Antibody Status	HPV Type Acquired Subsequently	No. of Persons At Risk	No. of Incident Cases	Person-months	Incidence Rate (per 1000 person-months)	Incidence Rate Ratio (95% CI)	
Anti-HPV 16 antibody							
Seropositive	HPV 16	41	3	536	5.6	1.1	(0.3-4.0)
Seronegative		235	15	3076	4.9	1.0	
Seropositive	HPV 16-related types *	39	2	515	3.9	0.6	(0.1-2.6)
Seronegative		231	19	2996	6.3	1.0	
Seropositive	Other HPV types	30	11	353	31.1	1.2	(0.6-2.4)
Seronegative		176	52	2073	25.1	1.0	
Anti-HPV 18 antibody							
Seropositive	HPV 18	60	1	809	1.2	1.8	(0.2-20.2)
Seronegative		223	2	2966	0.7	1.0	
Seropositive	HPV 18-related types **	55	3	727	4.1	0.6	(0.2-2.0)
Seronegative		211	19	2776	6.8	1.0	
Seropositive	Other HPV types	41	15	465	32.3	1.3	(0.7-2.3)
Seronegative		168	49	1988	24.6	1.0	

Note. 95% CI: 95 percent confidence interval.
* HPV 16-related types include HPV 31, 33, 35, 52, 58 and 67.
** HPV 18-related types include HPV 39, 45, 59, 68 and 70.

CHAPTER 3:

THE SECOND MANUSCRIPT

HUMAN PAPILLOMAVIRUS (HPV) 6, 11, 16 AND 18 SEROPREVALENCE IS ASSOCIATED WITH AGE AND SEXUAL PRACTICE: RESULTS FROM THE MULTI-NATIONAL HPV INFECTION IN MEN STUDY (*HIM STUDY*)

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References (Max: 50): 49

ABSTRACT

Background. Few HPV serology studies have evaluated type-specific seroprevalence of all vaccine HPV types in men. The present study investigates seroprevalence of HPV 6, 11, 16 and 18, and associated risk factors in men residing in three countries (U.S., Mexico and Brazil).

Methods. Data from 1477 men aged 18-70 enrolled in the *HIM* Study were analyzed. Serum antibody testing was performed with virus-like particle-based ELISA. Potential risk factors were assessed for individual HPV types using Logistic regression.

Results. Overall, HPV-6, 11, 16 and 18 seroprevalence was 14.8%, 17.3%, 11.2% and 5.8%, respectively. Thirty-four percent of men were seropositive to ≥1 HPV types. When examined by sexual practice, 31.2% of men who had sex with women (MSW), 65.6% of men who had sex with men (MSM) and 59.4% of men who had sex with both men and women (MSMW) were seropositive to ≥1 HPV types. Seroprevalence increased with age among young-to-middle-aged men with significant upward age trends observed for HPV 11, 16 and 18. Men with multiple lifetime male anal sex partners were 2-4 times more likely to be HPV 6 or 11 seropositive, and 3-11 times more likely to be HPV 16 or 18 seropositive.

Conclusion. Our data indicate that exposure to vaccine HPV types was common in men, particularly among MSM and MSMW. The practice of same-sex anal sex appears to be the strongest determinant for seroprevalence of all vaccine HPV types.

Key Words: Serum antibodies, seroprevalence, risk factors, Human Papillomavirus, Men who have sex with men, Men who have sex with men and women, same-sex sexual intercourse

INTRODUCTION

HPV infections cause approximately 85% of squamous cell anal cancer, 50% of penile cancer, 33-72% of oropharyngeal cancers, and 10% of laryngeal cancers [14, 49, 52, 53]. High prevalence of genital HPV infection has been reported in recent studies [17-19] with 25.8-72.9% of HIV-negative adult men testing positive for genital HPV. Anal and oral HPV, though less common, is present in 8.0-16.6% [20-22], and 2.9-7.6% [23] of HIV-negative adult men, respectively. Compared to men who have sex with women (MSW), men who have sex with men (MSM) and men who have sex with men and women (MSMW) were several times more likely to be infected with genital, anal or oral HPV, and hence at increased risk for HPV-associated diseases and cancers [24-28, 54].

Serum antibodies elicited by natural HPV infection reflect cumulative exposures to HPV over time and across anatomic sites. Anti-HPV serum antibody IgG detected by VLP-based assays are type-specific [49, 55, 56]. There is typically a 6-12 month latency for detection of antibodies following HPV DNA detection, as observed in women [57, 58]. Antibodies appear to be stable over time and remain detectable even after a decade [59-62]. Not all individuals challenged by natural exposure to HPV develop antibody responses detectable by current serology assays. Approximately 30-40% of women with incident HPV 16 infection failed to demonstrate seropositivity months following DNA detection [57, 58]. Similar data in men are not yet available. Although issues like differential seroconversion rates following infection for different HPV types, and unknown longevity of serum antibodies, could limit faithful translation, HPV serology remains a useful means for estimating cumulative HPV exposures in a population. With the recent licensure of the quadrivalent HPV vaccine for use in males, information on seroprevalence of vaccine-type HPV in the general male population is needed to provide guidance for strategic planning of vaccination. To date, few studies have characterized seroprevalence of all HPV types targeted in current vaccines and investigated

associated risk factors in men [30, 31, 33-37, 63-65]. Using baseline data from a large natural history study of HPV, we determined the seroprevalence of HPV 6, 11, 16 and 18, respectively, and identified demographic and behavioral factors that were independently associated with individual HPV seroprevalence in men.

METHODS

Study Population. The present study included a subset of participants enrolled in the HPV Infection in Men Study (the *HIM Study*), a multi-national longitudinal study of HPV infection in men. Details of the cohort have been reported elsewhere [66]. In brief, 4074 healthy men were recruited in São Paulo, Brazil, Cuernavaca, Mexico, and Tampa, Florida, United States, between June 2005 and August 2009. Men were recruited from several population sources in Brazil including public health clinic attendees, partners of women participating in a natural history study of HPV, and the general population. In Mexico, men were recruited from employees and beneficiaries of a large government agency and military officials. In the U.S., men were recruited from the University of South Florida and the greater Tampa metropolitan area. Men were considered eligible if the following criteria were met: (a) 18–70 years of age; (b) residents of 3 study sites; (c) no prior diagnosis of penile or anal cancers; (d) no prior diagnosis of genital or anal warts; (e) no symptoms of a sexually transmitted infection (STI) or current treatment of an STI; (f) no concurrent participation in an HPV vaccine study; (g) no history of HIV or AIDS; (h) no history of imprisonment, homelessness or drug treatment during the past 6 months; and (i) willingness to comply with 10 scheduled visits every 6 months for 4 years with no plans to relocate in 4 years. The current analysis included 1477 men with available serology, genital HPV DNA results and survey information from the enrollment visit.

Study Protocol. At the enrollment visit, an extensive sexual history and health questionnaire was administered using Computer-Assisted Self-Interviewing (CASI) system to solicit information on participant socio-demographic characteristics, sexual

history, condom use, alcohol and tobacco consumption, and history of abnormal pap smears in female partners. A 10ml venous blood was collected for serum antibody testing and participant external genitalia were sampled for HPV DNA testing.

HPV Serum Antibody Testing. Serum antibodies to HPV types 6, 11, 16, and 18 were measured using VLP-based enzyme-linked, immunosorbent assay (ELISA) [67]. HPV 6, 11 and 18 VLPs were produced in insect cells from recombinant baculoviruses expressing the L1 major capsid protein of individual HPV types [68], and HPV 16 VLPs were produced in mammalian cells from plasmids expressing the L1 and L2 capsid proteins [69]. Specimens were tested in duplicate on separate plates, with retesting of specimens showing results exceeding a preset, acceptable coefficient of variation (CV) of 25%. Seropositivity was defined as an optical density (OD) value greater than the mean OD value plus 5 standard deviations, estimated using serum samples from children, 1 to 10 years of age, after exclusion of outliers. Quality control of the serology assays was assured by inclusion of laboratory-prepared positive and negative controls in each run of the assay.

HPV DNA Sampling and Testing. Three pre-wetted Dacron swabs were used to collect exfoliated skin cells from the penis and scrotum and later combined to form a single specimen. All specimens were stored at -70°C until PCR analyses and genotyping were conducted. DNA was extracted from exfoliated skin cell samples using the QIAamp DNA Mini Kit (QIAGEN, Valencia, CA) and tested for HPV DNA using polymerase chain reaction (PCR) for amplification of a fragment of the HPV L1 gene [70]. HPV genotyping was conducted using the Linear Array HPV Genotyping Protocol (Roche Diagnostics, Indianapolis, IN) to detect 37 genital HPV types: 6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51-56, 58, 59, 61, 62, 64, 66-73, 81-84, IS39, and CP6108 [41].

Statistical Analysis. Overall seroprevalence and seroprevalence by sexual practice, age, country of residence, lifetime number of female partners and male anal

sex partners were presented in Figure1. Chi-squared and Cochran-Armitage test were used to compare seroprevalence and test for trends across levels of categorical variables, respectively. Serum antibody titer levels among seropositive men, as measured by OD values, were summarized by median and Interquartile Range (IQR). OD values were compared across levels of individual factors using Wilcoxon Rank-sum and Kruskal-Wallis test for each HPV type. The association between potential risk factors and HPV 6, 11, 16 or 18 seroprevalence, treated as a dichotomous outcome, was evaluated on a type-specific basis using unconditional logistic regression and measured by Odds Ratio (OR) and its 95% confidence intervals (CI). Factors examined included (1) sociodemographic characteristics such as age, country of residence, race, ethnicity, marital status, and educational attainment; (2) lifestyle and behavioral factors including alcohol consumption, smoking, circumcision, age at first sexual intercourse, sexual practice, the number of recent and lifetime sex partners, frequency of sexual intercourse, condom use, and history of other STIs; and (3) corresponding HPV DNA status at the same visit. Variables that demonstrated statistical significance at 0.1 level in the simple regression models were included in the multivariable regression models. Likelihood ratio tests and backward selection procedures were applied for variable selection. Factors were retained in the multivariable models if *p* values of likelihood ratio test were ≤0.05. Potential pair-wise interactions were explored. Because of the exploratory nature of the present study, no adjustment for multiple testing was made.

RESULTS

Characteristics of the 1477 men included in the current analysis and the median and inter-quartile range (IQR) of OD values among men seropositive to HPV 6, 11, 16 and 18 are summarized in Table 3.1. Of the 1477 men, 546 (37.0%) were residents of Tampa, US, 440 (29.8%) residents of São Paulo, Brazil and 491 (33.2%) residents of Cuernavaca, Mexico. These men were predominantly under 35 years of age (62.4%),

single (unmarried) or cohabiting (57.2%), had college or higher education (52.1%), and uncircumcised (58.3%). 1247 (84.4%) men were identified as MSW, 64 (4.3%) as MSM, 64 (4.3%) as MSMW, and 76 (5.1%) as men who reported never having any type of sex, based on their responses to multiple survey questions concerning their recent and lifetime sexual behaviors (Table 3.1).

Overall, HPV 6, 11, 16 and 18 seroprevalence was 14.8%, 17.3%, 11.2% and 5.8%, respectively (Figure 3.1.A). A total of 499 (33.8%) men were seropositive to ≥1 HPV type, and only 8 (0.5%) men were seropositive to all four HPV types. Seroprevalence of HPV 6/11 was 25.7% and seroprevalence of HPV 16/18 was 14.8% for the entire cohort.

When examined by sexual practice, 31.2% of MSW, 65.6% of MSM, 59.4% of MSMW, and 29.0% of men who never had sex were seropositive to ≥1 HPV type; and 0.2% of MSW, 6.3% of MSM, 1.6% of MSMW, and 0% of men who never had sex were seropositive to all four HPV types. Seroprevalence of HPV 6/11 was 23.5% for MSW, 51.6% for MSM, 45.3% for MSMW, and 25.0% for men who reported never having sex; whereas seroprevalence of HPV 16/18 was 11.8% for MSW, 50.0% for MSM, 45.3% for MSMW, and 7.9% for men who never had sex (Figure 3.1.A).

The seroprevalence of HPV 6, 11, 16 and 18 increased with age among young-to-middle-aged men (Figure 3.1.B) with significant upward age trends observed for HPV 11, 16 and 18 seroprevalence. HPV 6 and 11 seroprevalence peaked at age 35-44 and declined in older age, while HPV 16 seroprevalence plateaued after peaking at age 35-44 and HPV 18 seroprevalence continued to rise in older men. Seroprevalence also varied significantly by country of residence (Figure 3.1.C). Brazilian men had the highest seroprevalence for all HPV types among the three countries, reaching 19.5%, 25.0%, 16.1% and 10.5%, respectively. Type-specific seroprevalence did not differ significantly by the number of lifetime female sex partners among MSW (Figure 3.1.D). In contrast,

there was a statistically significant, strong positive trend of seroprevalence associated with the number of lifetime male anal sex partners for each HPV type (Figure 3.1.E).

The median and inter-quartile range (IQR) of serum antibody levels among seropositive men, as measured by OD values, were 0.234 (IQR: 0.187-0.312) for HPV 6, 0.244 (IQR: 0.188-0.355) for HPV 11, 0.190 (IQR: 0.121-0.427) for HPV 16, and 0.172 (IQR: 0.128-0.295) for HPV 18 (Table 3.1). Serum antibody levels differed significantly by sexual practice for all HPV types, and by lifetime number of male anal sex partners for HPV 6, 11 and 16. In addition, HPV 6 antibody levels differed significantly by the number of recent new sex partners; HPV 16 antibody levels differed by age and lifetime number of female sex partners; and HPV 18 antibody levels differed by circumcision status.

In univariate analyses, common risk factors for seroprevalence of all four HPV types were age, country of residence, sexual practice, the number of lifetime male anal sex partners, and recent new sex partners of either sex (Table 3.2). Additional factors significantly associated with seroprevalence in univariate analyses included education and cigarette smoking for HPV 6 seroprevalence; ethnicity, race, marital status, education, cigarette smoking, circumcision, age at first sex, the number of lifetime female sex partners among MSW, frequency of recent vaginal sex, and concurrent detection of HPV 11 DNA for HPV 11 seroprevalence; race, marital status, recent condom use in anal sex, and the history of other STIs for HPV 16 seroprevalence; and education, cigarette smoking, circumcision, and the history of other STIs for HPV 18 seroprevalence.

In multivariable analyses, the number of lifetime male anal sex partners was independently associated with seroprevalence of HPV 6, 11, 16 and 18, respectively (Table 3.3). Men with multiple lifetime male anal sex partners were more likely to be seropositive to HPV 6, 11, 16 or 18 [Adjusted Odds Ratio (AOR) for ≥11 partners: 4.34

(95% CI: 2.28-8.29) for HPV 6; 2.32 (95% CI: 1.12-4.84) for HPV 11; 7.74 (95% CI: 3.96-15.12) for HPV 16; and 11.46 (95% CI: 5.40-24.34) for HPV 18]. Increasing age was significantly associated with higher seroprevalence of HPV 11, 16 and 18, with an AOR of 1.50-1.58 for HPV 11, 1.81-2.23 for HPV 16 and 1.62-3.21 for HPV 18. In addition, for HPV 6, men with college or higher education had a significantly lower seroprevalence [AOR: 0.59 (95% CI: 0.41-0.85)]. For HPV 11, men with a recent new sex partner were significantly more likely to test seropositive [AOR: 1.64 (95% CI: 1.17-2.30)], whereas college-educated [AOR: 0.61 (95% CI: 0.43-0.88)] and circumcised men [AOR: 0.67 (95% CI: 0.48-0.94)] were less likely to test seropositive. For HPV 16, married men had significantly lower seroprevalence [AOR: 0.54 (95% CI: 0.33-0.90)]. For HPV 18, Brazilian residency was significantly associated with higher seroprevalence [AOR: 2.09 (95% CI: 1.04-4.19)].

DISCUSSION

The present study characterized the seroprevalence of four HPV types targeted in the currently licensed HPV vaccines and determined factors associated with individual HPV seroprevalence in 1477 men residing in U.S., Mexico and Brazil. Our findings demonstrate that approximately a third of cohort participants have been exposed to ≥1 vaccine HPV types, and the risk of exposure was twice as high among MSM and MSMW. Age was significantly associated with seroprevalence of all vaccine HPV types except for HPV 6, and anal sex with men was significantly associated with a higher risk of seropositivity to individual HPV types.

Age has been consistently associated with HPV seroprevalence in previous serology studies in men using VLP-based ELISA [30, 31, 33-37, 63-65, 71]. The upward age trends associated with HPV 16 and 18 seroprevalences observed in the current study are consistent with those reported in population-based [65], community-based [63, 71] or clinic-based studies [33, 35-37]. Only few studies have evaluated HPV 6 and/or 11

seroprevalence in men [30, 33, 64] and their findings on age have been mixed. The study of Hariri et al. showed a significant, increasing age trend for HPV 11 seroprevalence for males 6 to 49 years old followed by an insignificant decline through age 59 in a population-based study of 3589 men [64], similar to the age pattern observed for HPV 11 seroprevalence in the current study. In the remaining two studies, HPV 6 seroprevalence was significantly associated with older age (>35: OR, 2.2; 95% CI: 1.0-4.8) in the study of Hagensee et al. [30], whereas no age association with HPV 6/11 seroprevalence was detected by Slavinsky et al. [33]. The age patterns observed in the current study as well as previous studies likely reflect complex changes in individual immune capacity and the balance between HPV acquisition and clearance over their lifespan. The lack of age association in the study of Slavinsky et al [33] may be explained by the exclusive enrollment of highly sexually active STI clinic attendees who had multiple sex partners and a history of STI in the past year. It was also suggested that concomitant infection with other sexually transmitted agents may facilitate persistence of HPV infection, eliciting a stronger immune response and longer-lasting immune memory [34, 36, 37] and resulting in less variation in seroprevalence across age groups.

Our data demonstrated that seroprevalence of vaccine HPV types differed significantly between MSW, MSM and MSMW. To our knowledge, this study is the first to compare vaccine-type HPV seroprevalence between men with different sexual practices. Overall, MSM and MSMW exhibited approximately 2-6 times higher seroprevalence compared to MSW. No statistically significant trend or association of HPV seroprevalence with the number of lifetime female sex partners was observed among MSW for any vaccine HPV type examined. In contrast, seroprevalence increased significantly with increasing number of lifetime male anal sex partners for individual HPV types, and significant independent associations were detected for men reporting multiple

male anal sex partners. The higher seroprevalence associated with the practice of same-sex anal sex is consistent with previous findings [31, 34, 64, 65], and is likely explained by the increased risk of simultaneous HPV infections (oral, genital and anal) at multiple anatomic sites among MSM and MSMW. It probably also implies that HPV infection at keratinized epithelium is less likely to induce an immune response than infection of mucosal epithelium. In addition, it is noteworthy that seroprevalence of vaccine HPV types among men who reported never having any type of sex was comparable to that reported for MSW, except for HPV 16 seroprevalence which was much lower among those with no sex. It is possible that besides sexual intercourse, HPV transmission may have occurred through other forms of contact such as skin contact and use of sex toys, or through vertical transmission although less likely.

In the current study, circumcision was independently associated with lower HPV 11 seroprevalence but not with any other vaccine HPV types. A statistically significant association with circumcision was also observed in univariate analysis of HPV 18 seroprevalence. However, it was not retained in multivariate analyses. An approximately 30% reduction in acquisition and 30% increase in clearance of oncogenic HPV infection was demonstrated in recent randomized-controlled trials of circumcision [72, 73]. In addition, 2-3 times higher likelihood of clearance for both oncogenic and non-oncogenic HPV infections was shown in longitudinal epidemiological studies [40, 74]. It is possible that the lower incidence and shorter duration of HPV 11 infection due to circumcision may have contributed to the lower HPV 11 seroprevalence observed among circumcised men.

It was noted that in the present study detection of anti-HPV serum antibodies was not significantly associated with simultaneous presence of HPV DNA for any vaccine-type HPV, indicating a possible lag time between HPV DNA detection and detection of serum antibodies, and the limited value of serum antibodies as a marker of

current infection, consistent with what has been reported previously [58, 60, 75]. Our data also demonstrated substantial gaps between HPV DNA prevalence and seroprevalence. The gap was most prominent for HPV 11 (seroprevalence: DNA prevalence ratio=11.6) and least prominent for HPV 16 (seroprevalence: DNA prevalence ratio=1.8). It was suggested that longer presence of HPV DNA is associated with higher likelihood of seroconversion [57, 60]. With a longer duration of infection for HPV 16 than HPV 11 in this cohort of men [54], a smaller gap between HPV 16 DNA prevalence and seroprevalence was counterintuitive. However, HPV 16 and other oncogenic HPV were shown to have evolved an immune evasion mechanism inhibiting host detection of virus [76]. It was likely that the lack of immune evasion mechanism for HPV 11 and frequent presence of HPV 11-positive warts may have contributed to a higher seroconversion rate for HPV 11 than HPV 16. In addition, HPV 11 seroprevalence was associated with the presence of new sex partner in past 6 months, which may indicate a shorter latency period for HPV 11 serum antibody detection. The combination of a shorter latency and a higher seroconversion may have led to the great discrepancy observed between HPV 11 DNA prevalence and seroprevalence. Unique to HPV 6 and 11, a higher level of education was significantly associated with lower seroprevalence, which was also observed in the study of Hariri et al [64]. We hypothesize that education is likely a marker of lifestyle characteristics which may impact potential HPV transmission.

The major strengths of this study are the inclusion of men of a wide age range (18-70), especially those 60 and older, the age group that was rarely evaluated in previous studies; and the availability of detailed and extensive participant sexual behavioral information that has been previously validated [77]. However, several limitations must be addressed. First, this study is a cross-sectional analysis utilizing serology measured at the baseline of a large natural history study in men. With our

limited understanding of seroconversion rates following natural infection and the longevity of type-specific serum antibodies, baseline serostatus may underestimate the true proportion of cumulative HPV exposure, thus making it less informative for estimating population exposure. Second, differences in serological assays used, and the choice of control population and cutoff points for determining seropositivity between the present study and the previous studies may limit a direct comparison of seroprevalence in our study with those published previously. Finally, because of the recruitment method used in the present study, the cohort presented may not be a representative sample of the general male population of the participating country, which limits the generalizability of our findings.

Despite the aforementioned limitations, the present study provided important data on the distribution of vaccine-type HPV exposure in community-based male populations from three countries, and epidemiological factors associated with seroprevalence of individual HPV types targeted in the current vaccines. Our results indicated exposure to vaccine HPV types is common in men and increased from young to middle-aged men. Our data also demonstrated that MSM and MSMW had greatly elevated risk of exposure to vaccine HPV types compared to MSW, and the engagement in same-sex anal sex was a significant determinant of seroprevalence for all vaccine HPV types.

Table 3.1 Participant characteristics and serum antibody titers for HPV 6, 11, 16 and 18 among seropositive men.

Characteristics	No. (%)	HPV 6 antibody (OD, 10^{-3})		HPV 11 antibody (OD, 10^{-3})		HPV 16 antibody (OD, 10^{-3})		HPV 18 antibody (OD, 10^{-3})	
		median	IQR	median	IQR	median	IQR	median	IQR
Overall	1477	234	(187-312)	244	(188-355)	190	(121-427)	172	(128-295)
Age, *years*									
Median (range)	30 (18-78)								
18-24	477 (32.3)	233	(186-292)	239	(182-307)	134	(121-256)	259	(162-337)
25-34	444 (30.1)	244	(186-308)	244	(190-370)	161	(112-382)	164	(112-330)
35-44	375 (25.4)	221	(192-345)	272	(187-370)	260	(149-552)	172	(133-263)
45-70	181 (12.3)	260	(186-324)	230	(192-303)	203	(115-464)	154	(117-248)
p value[†]		0.943		0.528		0.035		0.409	
Country of residence									
US	546 (37.0)	218	(186-282)	242	(195-312)	177	(119-495)	174	(114-348)
Brazil	440 (29.8)	244	(192-324)	261	(192-423)	241	(121-445)	201	(133-326)
Mexico	491 (33.2)	242	(187-312)	239	(184-303)	195	(122-381)	154	(117-222)
p value[†]		0.338		0.154		0.849		0.363	
Marital Status									
Single	679 (46.0)	242	(186-324)	257	(192-368)	193	(122-427)	229	(128-421)
Cohabiting	166 (11.2)	245	(202-308)	205	(174-340)	160	(124-276)	192	(111-275)
Married	499 (33.8)	211	(185-287)	244	(188-340)	146	(119-397)	169	(137-242)
Divorced/Separated/	119 (8.1)	288	(217-364)	264	(207-486)	377	(130-544)	131	(112-149)

Characteristics	No. (%)	HPV 6 antibody (OD, 10^{-3})		HPV 11 antibody (OD, 10^{-3})		HPV 16 antibody (OD, 10^{-3})		HPV 18 antibody (OD, 10^{-3})	
		median	IQR	median	IQR	median	IQR	median	IQR
Widowed									
p value[†]		0.146		0.254		0.285		0.152	
Education									
< High school	335 (22.7)	245	(195-328)	224	(184-366)	155	(124-270)	150	(128-255)
High school graduate	357 (24.2)	265	(210-368)	255	(196-383)	191	(121-382)	153	(112-311)
≥College/ Vocational school	770 (52.1)	212	(182-284)	242	(191-308)	224	(121-495)	200	(136-302)
p value[†]		0.002		0.460		0.515		0.413	
Circumcision[a]									
No	861 (58.3)	244	(192-317)	246	(187-368)	172	(119-382)	175	(135-314)
Yes	616 (41.7)	222	(185-289)	242	(195-317)	241	(125-464)	141	(112-205)
p value[†]		0.077		0.999		0.304		0.031	
Sexual practice									
Sex with women	1247 (84.4)	222	(186-294)	230	(184-305)	146	(116-362)	161	(114-242)
Sex with men	64 (4.3)	318	(239-462)	296	(259-436)	368	(164-603)	398	(154-515)
Sex with both men and women	64 (4.3)	255	(210-328)	408	(249-626)	347	(153-672)	205	(133-255)
Never had sex	76 (5.1)	191	(169-306)	307	(209-371)	134	(122-145)	143	(125-163)
p value[†]		0.003		0.000		0.002		0.025	

Lifetime female sex partners among MSW, *no.*

Characteristics	No. (%)	HPV 6 antibody (OD, 10^{-3})		HPV 11 antibody (OD, 10^{-3})		HPV 16 antibody (OD, 10^{-3})		HPV 18 antibody (OD, 10^{-3})	
		median	IQR	median	IQR	median	IQR	median	IQR
None	76 (5.1)	188	(169-306)	317	(208-374)	134	(122-145)	143	(125-163)
1-3	303 (20.5)	236	(186-301)	230	(189-301)	124	(109-165)	161	(113-295)
4-7	285 (19.3)	250	(197-317)	223	(182-293)	139	(120-292)	114	(112-192)
8-16	277 (18.8)	222	(186-282)	221	(180-292)	149	(122-313)	174	(152-271)
≥17	280 (19.0)	211	(185-297)	258	(187-373)	269	(130-587)	123	(111-198)
p value†		0.587		0.280		0.018		0.096	
Lifetime male anal sex partners, *no.*									
None	1226 (83.0)	222	(186-299)	230	(187-310)	145	(116-338)	152	(114-222)
1	57 (3.9)	230	(186-268)	293	(184-450)	179	(122-390)	139	(139-139)
2-3	47 (3.2)	199	(182-292)	340	(258-671)	317	(155-479)	205	(169-271)
4-10	41 (2.8)	277	(218-503)	329	(261-563)	407	(241-603)	255	(136-435)
≥11	42 (2.8)	324	(222-376)	273	(241-449)	396	(160-737)	274	(125-409)
p value†		0.024		0.006		0.001		0.280	
Recent new sex partners (either sex), *no*									
None	972 (65.8)	217	(186-287)	240	(185-306)	159	(122-383)	157	(114-225)
1	318 (21.5)	266	(200-350)	258	(197-368)	214	(115-427)	198	(114-295)
≥2	165 (11.2)	261	(209-376)	302	(205-449)	224	(142-607)	309	(146-422)
p value†		0.011		0.155		0.465		0.055	

Note. Cells that do not add up to 100 percent are due to missing values.
OD: optical density used as measurement of serum antibody levels. IQR: Inter-quartile range. MSW: Men who have sex with women.
a. Circumcision status was assessed by study clinician. † p values were derived from Wilcoxon rank-sum or Kruskal-wallis test statistics

Table 3.2 Factors associated with seroprevalence of HPV 6, 11, 16 and 18 among 1477 men in univariate analyses.

Risk factors	HPV 6			HPV 11			HPV 16			HPV 18		
	sero-prevalence (%)	OR (95% CI)		sero-prevalence (%)	OR (95% CI)		sero-prevalence (%)	OR (95% CI)		sero-prevalence (%)	OR (95% CI)	
Age, *years*												
18-24	12.8	1.00		11.5	1.00		7.8	1.00		2.5	1.00	
25-34	14.4	1.15	(0.79-1.68)	19.8	1.90	(1.32-2.73) *	12.2	1.65	(1.06-2.56) *	6.1	2.51	(1.26-5.01) *
35-44	19.2	1.62	(1.12-2.35) *	21.9	2.15	(1.48-3.12) *	13.9	1.91	(1.23-2.99) *	7.5	3.13	(1.57-6.23) *
45-70	11.6	0.90	(0.53-1.52)	17.1	1.59	(0.98-2.56)	12.7	1.73	(0.99-3.00)	10.5	4.54	(2.16-9.56) *
Country of residence												
US	11.9	1.00		7.5	1.00		10.1	1.00		2.7	1.00	
Brazil	19.5	1.80	(1.27-2.55) *	25.0	4.10	(2.79-6.03) *	16.1	1.72	(1.18-2.50) *	10.5	4.13	(2.27-7.51) *
Mexico	13.6	1.17	(0.81-1.69)	21.4	3.35	(2.28-4.92) *	8.1	0.79	(0.52-1.21)	5.1	1.90	(0.99-3.64)
Hispanic												
No	15.2	1.00		15.5	1.00		12.0	1.00		5.5	1.00	
Yes	14.5	0.95	(0.71-1.27)	19.7	1.34	(1.02-1.76) *	10.0	0.81	(0.58-1.13)	6.0	1.09	(0.70-1.70)
Race												
White	15.8	1.00		13.6	1.00		12.4	1.00		6.6	1.00	
Black	15.7	1.00	(0.65-1.53)	21.4	1.73	(1.16-2.58) *	14.8	1.23	(0.78-1.92)	6.7	1.00	(0.54-1.87)
Other	13.7	0.85	(0.62-1.17)	20.0	1.59	(1.17-2.15) *	8.6	0.67	(0.46-0.97) *	4.4	0.65	(0.39-1.07)
Marital Status												
Single	14.9	1.00		15.0	1.00		11.6	1.00		5.0	1.00	

Risk factors	HPV 6			HPV 11			HPV 16			HPV 18		
	sero-prevalence (%)	OR (95% CI)		sero-prevalence (%)	OR (95% CI)		sero-prevalence (%)	OR (95% CI)		sero-prevalence (%)	OR (95% CI)	
Cohabiting	16.9	1.16	(0.73-1.84)	21.7	1.57	(1.02-2.40) *	14.5	1.28	(0.78-2.10)	6.6	1.35	(0.67-2.72)
Married	14.6	0.98	(0.71-1.36)	20.2	1.44	(1.06-1.94) *	7.8	0.64	(0.43-0.96) *	6.6	1.34	(0.82-2.20)
Divorced/Separated/Widowed	11.8	0.76	(0.42-1.39)	11.8	0.75	(0.42-1.37)	18.5	1.72	(1.03-2.89) *	5.0	1.01	(0.41-2.45)
Education												
< High school	20.3	1.00		23.9	1.00		9.6	1.00		8.7	1.00	
High school graduate	13.7	0.62	(0.42-0.93) *	19.9	0.79	(0.55-1.14)	9.5	1.00	(0.60-1.66)	5.0	0.56	(0.31-1.03)
≥ College/ Vocational school	13.0	0.59	(0.42-0.82) *	13.4	0.49	(0.36-0.68) *	12.7	1.38	(0.91-2.10)	4.9	0.55	(0.33-0.90) *
Alcohol drinking [a]												
Light drinking	16.6	1.00		18.7	1.00		10.3	1.00		5.2	1.00	
Moderate drinking	14.0	0.82	(0.56-1.21)	17.6	0.93	(0.65-1.33)	12.1	1.20	(0.78-1.86)	3.6	0.67	(0.34-1.33)
Heavy drinking	15.1	0.90	(0.59-1.37)	14.7	0.75	(0.49-1.15)	11.0	1.08	(0.66-1.78)	7.3	1.43	(0.77-2.68)
Cigarette smoking [b]												
Never	12.5	1.00		14.5	1.00		9.5	1.00		3.7	1.00	
Current, light-moderate	15.6	1.30	(0.74-2.28)	21.8	1.64	(0.99-2.72)	7.5	0.77	(0.37-1.60)	3.4	0.91	(0.31-2.68)
Current, heavy	20.4	1.79	(1.10-2.92) *	21.4	1.60	(1.00-2.56) *	12.9	1.42	(0.81-2.51)	7.0	1.94	(0.86-4.36)
Former, light-moderate	12.2	0.97	(0.51-1.85)	17.1	1.21	(0.69-2.14)	11.4	1.23	(0.62-2.42)	2.4	0.65	(0.18-2.36)
Former, heavy	16.2	1.36	(0.74-2.50)	13.5	0.92	(0.49-1.73)	13.5	1.50	(0.77-2.92)	9.0	2.57	(1.06-6.22) *

Risk factors	HPV 6			HPV 11			HPV 16			HPV 18		
	sero-prevalence (%)	OR (95% CI)		sero-prevalence (%)	OR (95% CI)		sero-prevalence (%)	OR (95% CI)		sero-prevalence (%)	OR (95% CI)	
Circumcision [c]												
No	16.0	1.00		21.5	1.00		11.3	1.00		7.0	1.00	
Yes	13.0	0.78	(0.58-1.05)	11.5	0.48	(0.35-0.64) *	11.2	0.99	(0.72-1.38)	4.2	0.59	(0.37-0.94) *
Sexual practice												
Sex with women	13.5	1.00		16.0	1.00		9.0	1.00		3.9	1.00	
Sex with men	34.4	3.36	(1.96-5.78) *	31.3	2.39	(1.38-4.15) *	40.6	6.93	(4.06-11.84) *	23.4	7.48	(3.93-14.27) *
Sex with both men and women	25.0	2.14	(1.19-3.86) *	31.3	2.39	(1.38-4.15) *	35.9	5.69	(3.29-9.82) *	26.6	8.84	(4.74-16.50) *
Never had sex	13.2	0.97	(0.49-1.93)	17.1	1.09	(0.59-2.01)	2.6	0.27	(0.07-1.13)	5.3	1.36	(0.48-3.87)
Age at first sexual intercourse, *years*												
Never had vaginal sex	13.1	0.96	(0.46-2.00)	20.8	2.12	(1.04-4.33) *	12.3	0.78	(0.38-1.61)	7.7	1.32	(0.49-3.59)
≤15	14.3	1.06	(0.58-1.93)	20.6	2.09	(1.12-3.90) *	12.8	0.81	(0.46-1.46)	7.3	1.24	(0.53-2.91)
16-17	14.9	1.12	(0.62-2.01)	14.9	1.41	(0.75-2.66)	10.0	0.62	(0.34-1.11)	4.7	0.78	(0.32-1.87)
18-20	17.6	1.36	(0.75-2.48)	17.6	1.73	(0.91-3.29)	9.4	0.58	(0.31-1.08)	5.5	0.92	(0.37-2.26)
≥21	13.6	1.00		11.0	1.00		15.3	1.00		5.9	1.00	
Lifetime female sex partners among MSW, *no.*												
None	14.5	1.26	(0.61-2.60)	15.8	1.27	(0.63-2.56)	2.6	0.29	(0.07-1.24)	5.3	1.47	(0.46-4.77)
1-3	11.9	1.00		12.9	1.00		8.6	1.00		3.6	1.00	
4-7	13.3	1.14	(0.70-1.86)	14.7	1.17	(0.73-1.87)	7.7	0.89	(0.49-1.61)	2.5	0.67	(0.26-1.75)

Risk factors	HPV 6			HPV 11			HPV 16			HPV 18		
	sero-prevalence (%)	OR (95% CI)		sero-prevalence (%)	OR (95% CI)		sero-prevalence (%)	OR (95% CI)		sero-prevalence (%)	OR (95% CI)	
8-16	15.5	1.36	(0.85-2.19)	17.3	1.42	(0.90-2.24)	8.7	1.01	(0.57-1.81)	5.1	1.41	(0.63-3.17)
≥17	13.9	1.20	(0.74-1.95)	18.9	1.58	(1.01-2.48) *	11.0	1.33	(0.77-2.30)	5.0	1.40	(0.62-3.13)
Lifetime male anal sex partners, *no.*												
None	13.3	1.00		15.7	1.00		8.3	1.00		4.0	1.00	
1	17.5	1.39	(0.69-2.80)	19.3	1.29	(0.66-2.53)	14.0	1.80	(0.83-3.90)	1.8	0.43	(0.06-3.16)
2-3	21.3	1.76	(0.86-3.61)	31.9	2.52	(1.34-4.75) *	23.4	3.37	(1.66-6.81) *	10.6	2.86	(1.08-7.55) *
4-10	29.3	2.70	(1.35-5.39) *	26.8	1.97	(0.97-4.01)	41.5	7.81	(4.06-15.00) *	22.0	6.76	(3.06-14.93) *
≥11	40.5	4.43	(2.34-8.39) *	31.0	2.41	(1.23-4.73) *	47.6	10.02	(5.29-18.97) *	38.1	14.78	(7.45-29.33) *
Recent new sex partners (either gender), *no*												
None	13.4	1.00		15.6	1.00		9.6	1.00		4.5	1.00	
One	18.2	1.45	(1.03-2.03) *	21.7	1.50	(1.09-2.05) *	12.3	1.32	(0.89-1.97)	6.0	1.34	(0.77-2.33)
Two or more	16.4	1.27	(0.81-1.99)	18.2	1.20	(0.78-1.85)	17.0	1.93	(1.22-3.06) *	12.1	2.91	(1.67-5.08) *
Frequency of recent vaginal sex, times/week												
<1	14.5	1.00		14.9	1.00		9.4	1.00		4.4	1.00	
1-2	14.2	0.98	(0.65-1.49)	21.2	1.54	(1.05-2.24) *	6.9	0.72	(0.41-1.24)	5.6	1.28	(0.66-2.49)
≥3	16.1	1.13	(0.77-1.68)	19.5	1.39	(0.95-2.01)	10.5	1.13	(0.71-1.81)	4.0	0.91	(0.45-1.85)
Recent condom use in vaginal sex												
Always	11.8	1.00		16.7	1.00		9.4	1.00		3.5	1.00	

Risk factors	HPV 6			HPV 11			HPV 16			HPV 18		
	sero-prevalence (%)	OR (95% CI)		sero-prevalence (%)	OR (95% CI)		sero-prevalence (%)	OR (95% CI)		sero-prevalence (%)	OR (95% CI)	
Frequently	13.6	1.17	(0.71-1.92)	18.6	1.14	(0.74-1.76)	7.9	0.82	(0.46-1.49)	3.9	1.14	(0.48-2.72)
Sometimes	17.6	1.60	(0.92-2.77)	15.0	0.88	(0.52-1.52)	11.8	1.29	(0.69-2.42)	4.6	1.33	(0.50-3.57)
Never	16.0	1.43	(0.92-2.20)	18.8	1.16	(0.79-1.70)	10.1	1.09	(0.66-1.79)	6.5	1.95	(0.94-4.03)
Recent condom use in anal sex												
Always	26.4	1.00		27.8	1.00		23.6	1.00		12.5	1.00	
Frequently	21.5	0.76	(0.40-1.47)	22.8	0.77	(0.40-1.45)	15.2	0.58	(0.28-1.20)	10.1	0.79	(0.33-1.91)
Sometimes	23.8	0.87	(0.39-1.94)	28.6	1.04	(0.49-2.23)	19.0	0.76	(0.32-1.80)	9.5	0.74	(0.24-2.31)
Never	23.7	0.87	(0.53-1.41)	23.2	0.78	(0.48-1.28)	10.6	0.38	(0.21-0.69) *	8.2	0.63	(0.31-1.26)
History of other STIs [d]												
No	14.1	1.00		16.5	1.00		9.8	1.00		4.9	1.00	
Yes	18.3	1.36	(0.95-1.96)	21.5	1.39	(0.99-1.96)	18.3	2.07	(1.42-3.01) *	10.2	2.19	(1.34-3.56) *
HPV DNA status												
Negative	14.5	1.00		17.0	1.00		11.0	1.00		5.8	1.00	
Positive	18.8	1.37	(0.81-2.31)	36.4	2.78	(1.15-6.70) *	15.2	1.46	(0.80-2.64)	7.4	1.30	(0.30-5.59)

Note. CI: Confidence interval. * Denote statistical significance ($p<0.05$)
a. Light drinking was defined as <half a drink per day; moderate drinking as one half to 2 drinks per day; and heavy drinking as >2 drinks per day on average.
b. Heavy exposure was defined as ≥913 pack-years, and light-moderate exposure as <913 pack-years, equivalent to 10 cigarettes per days for 5 years.
c. Circumcision status was assessed by study clinician.
d. MSW: Men who have sex with women.
e. Other STIs: Chlamydia, HSV, genital warts, Gonorrhea, Hepatitis B, Hepatitis C, non-gonococcal urethratitis, Syphilis.

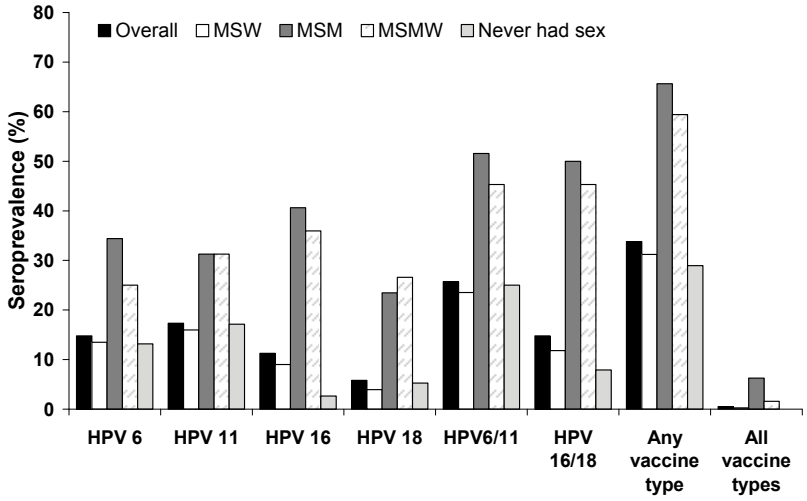
Table 3.3 Factors associated with seroprevalence of HPV 6, 11, 16 and 18 among 1477 men in multivariable analyses.

Characteristics	HPV 6		HPV 11		HPV 16		HPV 18	
	AOR (95% CI)		AOR (95% CI)		AOR (95% CI)		AOR (95% CI)	
Age, *years*								
18-24	1.00		1.00		1.00		1.00	
25-34	0.95	(0.64-1.41)	1.50	(1.03-2.23)*	1.81	(1.09-3.01)*	1.62	(0.75-3.46)
35-44	1.31	(0.88-1.93)	1.58	(1.06-2.37)*	2.22	(1.26-3.92)*	2.13	(1.00-4.52)*
45-70	0.76	(0.44-1.31)	1.44	(0.87-2.39)	2.23	(1.13-4.40)*	3.21	(1.41-7.33)*
Country of residence								
US	--		--				1.00	
Brazil	--	--	--	--	--	--	2.09	(1.04-4.19)*
Mexico	--	--	--	--	--	--	1.63	(0.81-3.33)
Marital Status								
Single	--		--		1.00		--	
Cohabiting	--	--	--	--	1.19	(0.68-2.08)	--	--
Married	--	--	--	--	0.54	(0.33-0.90)*	--	--
Divorced/Separated/Widowed	--	--	--	--	1.20	(0.64-2.25)	--	--
Education								
< High school	1.00		1.00		--		--	
High school graduate	0.67	(0.44-1.02)	0.89	(0.61-1.31)	--	--	--	--
≥ College/ Vocational school	0.59	(0.41-0.85)*	0.61	(0.43-0.88)*	--	--	--	--
Circumcision [a]								
No	--		1.00		--		--	

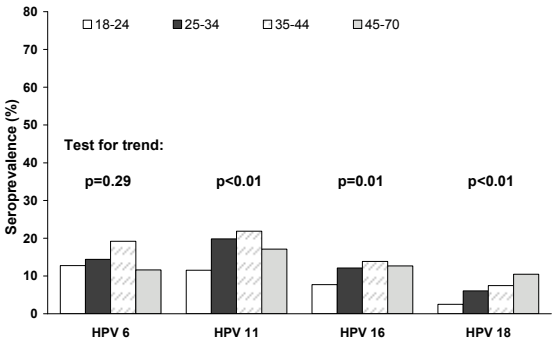
Yes	--	--	0.67	(0.48-0.94)*	--	--	--	--
Lifetime male anal sex partners, *no.*								
None	1.00		1.00		1.00		1.00	
1	1.32	(0.65-2.67)	1.24	(0.62-2.47)	1.61	(0.74-3.54)	0.35	(0.05-2.57)
2-3	1.68	(0.82-3.47)	2.14	(1.13-4.09)*	3.34	(1.63-6.85)*	2.21	(0.81-6.03)
4-10	2.68	(1.33-5.41)*	1.99	(0.96-4.15)	7.19	(3.68-14.05)*	6.10	(2.67-13.94)*
≥11	4.34	(2.28-8.29)*	2.32	(1.12-4.84)*	7.74	(3.96-15.12)*	11.46	(5.40-24.34)*
Recent new sex partners (either sex), *no*								
None	--		1.00		--		--	
1	--	--	1.64	(1.17-2.30)*	--	--	--	--
≥2	--	--	1.01	(0.61-1.66)	--	--	--	--

Note. AOR: Adjusted Odds Ratio. CI: Confidence interval. * Denote statistical significance ($p<0.05$)
a. Circumcision status was assessed by study clinician.

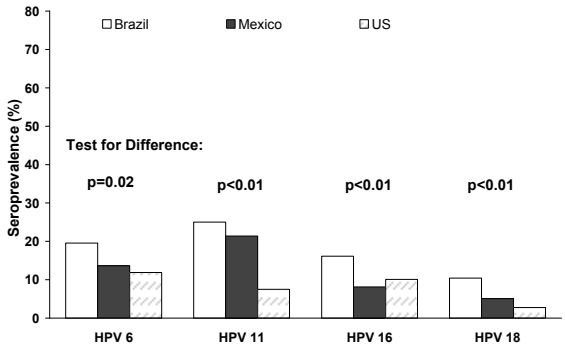
A. Seroprevalence overall and by sexual practice.



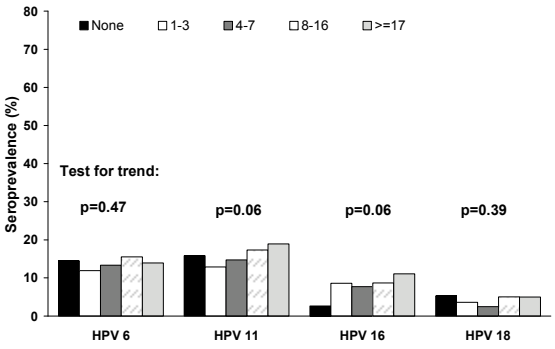
B. Seroprevalence by age.



C. Seroprevalence by country of residence.



D. Seroprevalence by lifetime female partners.



E. Seroprevalence by lifetime male anal sex partners.

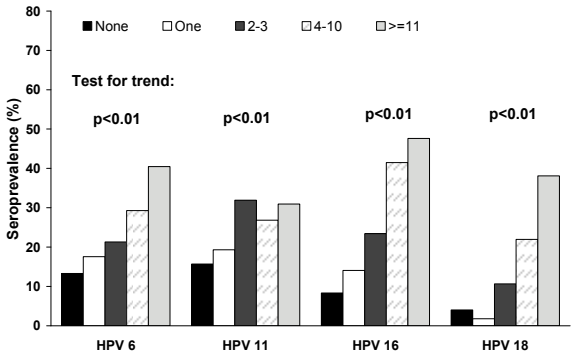


Figure 3.1 Seroprevalences of HPV 6, 11, 16 and 18 overall and by sexual practice, age, country, and lifetime number of female and male sex partners. (A) Seroprevalence overall and by sexual practice; (B) Seroprevalence by age; (C) Seroprevalence by country of residence; (D) Seroprevalence by lifetime number of female sex partners among men who have sex with women (MSW); (E) Seroprevalence by lifetime number of male anal sex partners.

CHAPTER 4:

THE THIRD MANUSCRIPT

A PROSPECTIVE STUDY OF GENITAL HUMAN PAPILLOMAVIRUS (HPV) 16 INFECTION IN ASSOCIATION WITH BASELINE SERUM ANTIBODIES TO HPV 16

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Running Title: Baseline HPV 16 Serostatus and Genital HPV 16 Acquisition

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Tables and Figures (Max: 7 inserts): 4 tables

References (Max: 50): 30

Appendices: 3 tables

INTRODUCTION

Genital HPV infection is one of the most common sexually transmitted infections (STIs) in the United States [1]. Prevalence of up to 63% has been documented in U.S. men [17], with HPV 16 being the most frequently detected oncogenic HPV type [78, 79]. In women detectable anti-HPV 16 serum antibodies protect against subsequent genital HPV 16 infection and HPV 16-related CIN2+ in a population-based prospective study [80]. Whether a man's risk of acquiring genital HPV 16 infection is altered by the presence of anti-HPV 16 serum antibodies remains unclear. We have previously reported that the detection of HPV 16 seropositivity was not associated with risk of subsequent genital HPV 16 infection among a cohort of U.S. men [71]. However, that study had a limited duration of follow-up and a relatively small sample size. In addition, unavailability of quantitative measurement of serum antibody titers had limited our ability to assess potential associations between circulating serum antibody titers and the risk of infection.

Risk of HPV infection at different anatomic sites appear to differ between men who had sex with women (MSW), men who had sex with men (MSM) and men who had sex with both men and women (MSMW), with MSM and MSMW at increased risk of infection [26, 81]. There is also growing evidence that HPV infection acquired at various anatomic sites may differentially contribute to circulating antibody levels observed in men [31, 34, 64, 65, 82]. In previous HPV serology studies, men who had same-sex sexual intercourse were more likely to have detectable antibodies to HPV types 6, 11, 16, or 18, compared to men who had sex with only women [31, 34, 64, 65, 82]. As a result, any potential protection conferred by detectable serum antibodies may differ between men with different sexual practices. In the current study we examined the risk of genital HPV 16 infection by enrollment serum antibody status in a large cohort of men, for the entire cohort and according to their sexual practices.

METHODS

Study Population. A multi-national longitudinal study of HPV infection in men (*HIM Study*) was conducted in Tampa, Florida, United States, São Paulo, Brazil, and Cuernavaca, Mexico. Enrollment to the cohort occurred between June 2005 and August 2009. Details of the cohort have been reported previously [66]. In brief, healthy men were recruited from several population sources in each study site and followed every six months for a maximum of 4 years. Men were eligible to participate if the following criteria were met: (a) 18–70 years of age; (b) residents of 3 study sites; (c) no prior diagnosis of penile or anal cancers; (d) no prior diagnosis of genital or anal warts; (e) no symptoms or current treatment for an STI; (f) no concurrent participation in an HPV vaccine study; (g) no history of HIV or AIDS; (h) no history of imprisonment, homelessness or drug treatment during the past 6 months; and (i) willingness to comply with 10 scheduled visits every 6 months for 4 years with no plans to relocate in 4 years. All eligible men signed an informed consent prior to enrollment. At the enrollment visit and each follow-up visit, an extensive sexual history and health questionnaire was administered using Computer-Assisted Self-Interviewing (CASI) system. 10ml venous blood was collected for serum antibody testing and the external genitalia were sampled for HPV testing. The informed consent and the study protocol were reviewed and approved by appropriate internal review boards and human subject committees at each study site.

A total of 4074 men residing in Tampa, São Paulo and Cuernavaca were enrolled in the *HIM Study*. The present analysis included a subset of 2187 men for whom HPV 16 serology and survey information was available from the baseline, and HPV 16 DNA results available from the baseline and at least one follow-up visit as of August 31st, 2010, and who tested negative for HPV 16 DNA at baseline. Comparison of participant characteristics indicated that the subset is comparable with the full study cohort of the

HIM Study with respect to socio-demographic characteristics, sexual behaviors and lifestyle factors, except that a slightly larger proportion of Brazilian participants were represented in the current cohort (Table 4.1).

HPV Serum Antibody Testing. Serum antibodies to HPV types 16 were measured using virus-like particle (VLP)-based enzyme-linked, immunosorbent assay (ELISA) [67]. HPV 16 VLPs were produced in insect cells from recombinant baculoviruses expressing HPV 16 L1 capsid proteins [68]. Specimens were tested in duplicate on separate plates, with retesting of specimens showing results exceeding a preset, acceptable coefficient of variation (CV) of 25%. Seroreactivity was measured by absorbance values, optical density (OD). The mean and standard deviation (SD) of absorbance values were estimated based on seroreactivity of serum samples from children, 1 to 10 years of age. The mean value plus 5 standard deviations was used as the cut point for seropositivity. Quality control of the serology assays was assured by inclusion of laboratory-prepared positive and negative controls in each run of the assay.

HPV DNA Sampling and Testing. Three pre-wetted Dacron swabs were used to collect exfoliated skin cells from the penis and scrotum and later combined to form a single specimen. All specimens were stored at -70°C until PCR analyses and genotyping were conducted. DNA was extracted from exfoliated skin cell samples using the QIAamp DNA Mini Kit (QIAGEN, Valencia, CA) and tested for HPV DNA using polymerase chain reaction (PCR) for amplification of a fragment of the HPV L1 gene [83]. HPV genotyping was conducted using the Linear Array HPV Genotyping Protocol (Roche Diagnostics, Indianapolis, IN) to detect 37 genital HPV types [41]. Human β-globin was tested to assure the integrity of DNA and was detected in 94.9% (2076/2187) of baseline HPV samples tested.

Statistical Analysis. Participants were classified as MSW (89.3%), MSM (5.0%), and MSMW (5.7%) based on their responses to multiple survey questions regarding

their recent and lifetime sexual behavior. As the number of MSM and MSMW was small, and both groups of men engaged in same-sex sexual behaviors, MSM and MSMW were combined and regrouped as MSM in the current analysis. Characteristics of seronegative and seropositive men were compared using Chi-squared and Fisher's Exact Test among the overall cohort, MSW and MSM, respectively. Two virological endpoints were evaluated: incidence of HPV 16 infection and incidence of 6-month persistent HPV 16 infection. An incident HPV 16 infection was defined as the first detection of HPV 16 DNA by the Linear Array assay during the follow-up period, assuming the date of detection as the date of occurrence. An incident 6-month persistent HPV 16 infection was defined as the detection of HPV 16 DNA at two or more consecutive visits during the follow-up period, using the date of first positive DNA detection as the date of occurrence. Incidence proportion of both endpoints for each study interval was summarized using the number of incident cases detected at the stated visit as the numerator and the number of participants who tested HPV 16 DNA negative at the prior visit and returned for the stated visit as the denominator. Cox proportional hazard regression was applied to compare risk of HPV 16 infection by participant baseline serostatus for the entire cohort, MSW and MSM, respectively, controlling for potential confounders. Serostatus was included in the Cox models as a binary variable (seropositive vs. seronegative) as well as a continuous variable measuring antibody titers, indicated by OD and log-transformed. Men who tested negative for HPV 16 DNA throughout their last available follow-up visit were considered censored at the last visit. Potential confounders considered included (1) socio-demographic characteristics such as age, country of residence, race, ethnicity, marital status, and educational attainment; and (2) lifestyle and behavioral factors including alcohol consumption, smoking, circumcision, age at first sexual intercourse, sexual practice, the number of recent and lifetime sex partners, frequency of sexual intercourse,

condom use, and history of other STIs. Individual factor that demonstrated statistical significance at 0.1 level along with serostatus in Cox models was considered for inclusion in the multivariable models. Partial likelihood ratio and Wald tests were used for covariate selection using a backward elimination procedure. Hazard Ratio (HR) and its 95% confidence intervals (CI) were estimated from Cox regression models. All 2187 men were included in the analysis of incident HPV 16 infection. Of the 2187 men, 1834 men with DNA results for at least two consecutive follow-up visits remained in the analysis for incident 6-month persistent HPV 16 infection.

RESULTS

The cohort of 2187 men contributed a total of 10086 visits, equivalent to 4424 person-years. 739 (33.8%) men were followed for approximately 12 months (2 follow-up visits), 728 (33.3%) for 24 months (4 visits), 559 (25.6%) for 36 months (6 visits), and 161 (7.4%) for 48 months (8 visits). The mean and median duration of follow-up was 2.0 years (range: 0.4-4.1; inter-quartile range: 1.2-3.0). The median interval between visits was 6.2 months.

Characteristics of seronegative men and seropositive men were summarized in Table 4.2. Baseline seroprevalence of HPV 16 was 12.3% overall, 10.0% among MSW and 31.2% among MSM. Overall, seronegative and seropositive men differed significantly by age at enrollment, country of residence, the number of lifetime sex partners (either sex) and new sex partners (either sex) in the past 6 months. Seropositive men were more likely to be 25 years or older, Brazilian, and have a large number of lifetime sex partners and one or more new sex partners in past 6 months. Similarly, significant differences were observed in age at enrollment, country of residence, and the number of lifetime sex partners (either sex) and lifetime female sex partners between seronegative and seropositive MSW. Among MSM, seropositive men were significantly older compared to seronegative men at enrollment.

Overall, a total of 221 (10.1%) men developed incident HPV 16 infections (seronegative 9.8% vs. seropositive 12.6%), and 72 (3.9%) men developed 6-month persistent HPV 16 infections (seronegative 3.9% vs. seropositive 3.8%) during the study period (Table 4.3). A smaller proportion of seropositive men had incident HPV 16 infections than seronegative men during the first year of follow-up (6 months: Overall 3.0 vs. 3.3%; MSW 3.1 vs. 3.2%; MSM 2.7 vs. 5.0%; 12 months: Overall 2.1 vs. 3.0%; MSW 1.8 vs. 2.8%; MSM 3.0 vs. 5.5%). The difference was more apparent among MSM. However, the reduction in HPV 16 incidence was not sustained through out the study period. Likewise, seropositive MSM had a lower incidence of 6-month persistent HPV 16 infection than seronegative MSM (6 months: 0 vs. 2.1%; 12 months: 1.5 vs. 2.8%) in the first year which was not retained for the remaining study period.

The risk of incident HPV 16 infection and 6-month persistent infection according to baseline serostatus were presented in Table 3. Overall, baseline serostatus was not associated with risk of incident HPV 16 infection in either univariate or multivariable analyses (HR: 1.23, 95% CI: 0.85-1.77; Adjusted HR: 1.22, 95% CI: 0.83-1.79) (Table 4.4.a). Likewise, incidence of 6-month persistent HPV 16 infection was not associated with baseline seropositivity in either univariate (HR: 0.96, 95% CI: 0.48-1.93) or multivariable analysis (Adjusted HR: 1.05, 95% CI: 0.51-2.16) (Table 4.4.b).

When we examined the same associations according to sexual practices, the risk of incident HPV 16 infection was not associated with baseline serostatus among MSM (Adjusted HR: 1.23, 95% CI: 0.60-2.50) or MSW (Adjusted HR: 1.60, 95% CI: 0.98-2.60) (Table 4.5.a). Insignificant associations were also detected in the analysis of 6-month persistent HPV 16 infection among MSM and MSW, respectively (Table 4.5.b). An insignificant negative association between baseline seropositivity and risk of 6-month persistent HPV 16 infection was observed among MSM in the univariate analysis (HR: 0.53, 95% CI: 0.11-2.51). However, the association was not confirmed in the

multivariable analysis (Adjusted HR: 1.03, 95% CI: 0.21-4.98). No protection against 6-month persistent HPV 16 infection was observed among MSW.

We further determined if higher serum antibody titers were associated with lower likelihood of subsequent HPV 16 infection. We did not observed significant associations between serum antibody titers and risk of incident HPV 16 infection and 6-month persistent HPV 16 infection for the overall cohort, MSW or MSM, respectively (Table 4.6.a-b). Nor did we observe significant associations after we further restricted the analysis to seropositive men only (data not shown).

DISCUSSION

In this multi-national prospective study of healthy men, we assessed the risk of genital HPV 16 infection over a 48-month study period by baseline HPV 16 serostatus to determine if anti-HPV 16 serum antibodies induced by prior infection confer protection against subsequent acquisition of HPV 16 infection. Our study is one of the few studies that investigate the potential protective role of serum antibodies in the natural history of genital HPV in men. Two endpoints were evaluated, respectively: incident HPV 16 infection and 6-month persistent HPV 16 infection. Overall the risk of incident HPV 16 infection appeared to be lower in seropositive men compared to seronegative men within the first year of study period, particularly among MSM. Likewise, MSM had a lower incidence of 6-month persistent infection within the first year. However, no statistically significant associations between baseline HPV 16 seropositivity and risk of incident HPV 16 infection or 6-month persistent HPV 16 infection over the entire study period were detected. Nor did we find that higher HPV 16 serum antibody titers were significantly associated with lower risk of infection.

While we were not able to demonstrate statistically significant differences in the risk of HPV 16 infection, our data showed that HPV 16 incidence was lower among seropositive men compared to seronegative men during the first year of follow-up,

became higher and remained higher among seropositive men in the months that follow. Previous studies in women have shown that in the absence of viral antigen, serum IgG antibody titers attenuate over time. In the prospective study of Shoultz et al [84], female STI clinic attendees were followed every 4 month for a median of 24.6 month for repeated assessment of serostatus using L1 VLP-based capture ELISA. Among 197 women with at least two consecutive HPV 16 seropositives during the study period, the median antibody titer was observed to decline by nearly 30-35% within 12-18 months following initial detection and to remain relatively stable over a long period of follow-up. Whereas, among 223 women who tested HPV 16 seropositive only once, the median antibody titer declined by approximately 70% in the first 6 months, remained below the positive cut-point for the remainder of the year, and rebound and fluctuated around positive cut-point throughout the study period [84]. Similarly, Ho et al reported cumulative probabilities of losing anti-HPV 16 IgG seropositivity by 12, 24 and 36 months were 38.5%, 40.0% and 48.2% among female university students who tested seropositive at least once during the study period by L1/ L2 VLP-based direct ELISA [58]. The loss of serum antibody titer over time is a possible explanation for our findings. This hypothesis needs to be tested in future studies where repeated assessments of serum antibody and DNA status are available.

Both the risk of incident HPV 16 infection and 6-month persistent HPV 16 infection was observed to be insignificantly associated with baseline HPV 16 serostatus or serum antibody titers in our cohort, which is consistent with what we found previously in men [71]. Findings from serology studies in women have been mixed [38, 39, 80, 85, 86]. Using L1/L2 VLP-based direct ELISA, Ho et al demonstrated that persistence of high anti-HPV 16 antibody titers at ≥2 consecutive visits was associated with lower risk of subsequent HPV 16 infection (Adjusted RR: 0.49; p=0.037) among female university students [38]. Wentzensen et al. showed a significant reduction in the risk of subsequent

HPV 16 infection or HPV 16-positive CIN2+ for seropositive women compared to seronegative women using a VLP-based ELISA (Adjusted OR: 0.60, 95% CI: 0.40-0.90) and a competitive Luminex-based immunoassay (cLIA) (Adjusted OR: 0.44, 95% CI: 0.21-0.93) in a nested case-control study of 974 participants of the Guanacaste Natural History Study [80]. In addition, a protective effect was observed by Velicer et al among women aged 24-34 enrolled in the placebo arm of a HPV vaccine trial using cLIA, as evidenced by a lower incidence of HPV 6/11/16/18 infection and 6-month persistent infection among baseline seropositive women compared to seronegative women (incident infection: 1.0 [0.31–2.23] vs. 5.7 [4.68–6.84] per 100 woman-years; 6-month persistent infection: 0.4 [0.05–1.37] vs. 2.5 [1.89–3.35] per 100 woman-years) [86]. In contrast, no protective effect was reported by Viscidi et al among 7,046 women enrolled in the Guanacaste cohort (RR: 0.74, 95% CI: 0.45–1.20) [39], or by Trottier et al among 1,902 women enrolled in the Ludwig-McGill cohort both using a L1/L2 VLP-based direct ELISA assay (Incidence: Lowest tertile of antibody titers 1.6 [95% CI: 1.3–2.1] vs. Highest tertile 2.1 [95% CI: 1.7–2.6] per 1000 woman-months) [85].

The lack of protection observed in the present study in contrast to the relatively strong protection observed in female studies could be due to several factors, including differential gender-related immune response, assay differences and varying persistence of serum antibodies. Gender differences in seroprevalence have been reported in multiple studies [29, 31, 34, 36, 37, 65]. Seroprevalence of oncogenic and non-oncogenic HPV was consistently higher among women than men from the same source population. The observed gender-specific immune response probably implies that HPV infection at keratinized epithelium is less likely to induce an immune response than infection of mucosal epithelium. Furthermore, differences in serological assays used across studies may explain the inconsistent results across publications. The VLP-based direct-binding ELISA assay used in the current study measures total type-specific

binding IgG antibodies, including neutralizing and non-neutralizing antibodies; whereas the competitive neutralization assay utilized by Wentzensen et al [80] and Velicer et al [86] measures IgG antibodies that bind to known neutralizing epitopes in HPV viral capsid. The inclusion of non-neutralizing antibodies in the present study could have led to overestimation of neutralizing antibody titers, and biased the association of protection toward null. Lastly, persistence of serum antibodies could have played a role in conferring protection. A protective effect of serum antibodies against incident infection with HPV 16 and its related types was observed among young women who had high antibody titer detected at ≥2 consecutive visits using a direct ELISA assay in the study of Ho et al [38]. In contrast, no protection was detected in other studies in women that measured serostatus only once at the baseline using similar assay [39, 86].

Among all incident HPV 16 infections that occurred over the study period, ~35% of cases in the seronegative group and ~24% of cases in the seropositive group were detected at the 6-month visit. It was unclear what proportion of these infections may in fact be reactivation of prior infections. In an attempt to distinguish the possible reactivated infection from the truly new infection, we evaluated the risk of incident and 6-month persistent infection with HPV 16 by baseline serostatus after restricting incident cases to those detected at the 12-month visit or later (Table 4.7.a-b). However, no significant association between baseline serostatus and risk of infection was observed in the restricted analysis.

The present study is unique in its longitudinal design, the size of the cohort, the long duration of follow-up achieved in a large proportion of the cohort, and the availability of repeated measurements of HPV 16 DNA status. Yet limitations of the study have to be addressed. As the serum antibody measurement was only obtained once at enrollment, with natural fluctuation or attenuation of serum antibody titer over time [45, 58], there was possible misclassification of baseline serostatus which could have driven

the association toward the null. Finally, despite the relatively large sample size for the present analysis, due to the low incidence of HPV 16 and the small proportion of MSM, our stratified analyses remained statistically underpowered to detect potential significant associations of HPV 16 acquisition with baseline serostatus among MSM.

In conclusion, our data indicated that the presence of anti-HPV 16 serum antibodies at baseline did not alter the risk of HPV 16 acquisition in men. Future studies that investigate the effect of serum antibodies on the acquisition and clearance of HPV infection taking into account the duration of serum antibody presence are necessary to provide a greater understanding of the role of serum antibodies in the natural history of HPV in men.

Table 4.1 Participant characteristics in the current cohort and in the full study cohort of the *HIM Study*.

Characteristics	Current Cohort (N=2187)	Full *HIM* Cohort (N=4074)	p values[†]
	n (%)	n (%)	
Age, years			0.513
18-24	643 (29.4)	1232 (30.2)	
25-44	1240 (56.7)	2315 (56.8)	
45-70	304 (13.9)	527 (12.9)	
Country			**0.003**
Brazil	819 (37.4)	1401 (34.4)	
Mexico	735 (33.6)	1330 (32.6)	
US	633 (28.9)	1343 (33.0)	
Marital			0.719
Single	975 (44.7)	1838 (45.2)	
Cohabiting	280 (12.8)	484 (11.9)	
Married	744 (34.1)	1384 (34.1)	
Divorced/Widowed	183 (8.4)	357 (8.8)	
Education			0.717
Less than high school	464 (21.3)	900 (22.2)	
High School graduate	593 (27.2)	1089 (26.8)	
College or higher	1124 (51.5)	2071 (51.0)	
Alcohol drinking [a]			0.722
Light	692 (43.2)	1281 (43.9)	
Moderate	574 (35.8)	1012 (34.6)	
Heavy	336 (21.0)	628 (21.5)	
Cigarette smoking			0.462
Never	455 (33.3)	845 (33.0)	
Current	489 (35.8)	963 (37.6)	
Former	422 (30.9)	751 (29.3)	
Circumcision [b]			0.070
No	1437 (65.7)	2583 (63.4)	
Yes	750 (34.3)	1491 (36.6)	
Sexual practice			0.798
MSW	1953 (89.3)	3429 (89.7)	
MSM	109 (5.0)	176 (4.6)	
MSMW	125 (5.7)	217 (5.7)	
Lifetime female sex partners among MSW, no.			0.597
None	2 (0.1)	3 (0.1)	
1-3	510 (27.8)	835 (26.1)	

Characteristics	Current Cohort (N=2187)	Full *HIM* Cohort (N=4074)	p values†
	n (%)	n (%)	
4-17	876 (47.8)	1574 (49.1)	
>=18	446 (24.3)	792 (24.7)	
Lifetime male anal sex partners, no.			0.366
None	1829 (85.1)	3480 (86.6)	
1-2	149 (6.9)	238 (5.9)	
3-10	96 (4.5)	168 (4.2)	
>=11	76 (3.5)	132 (3.3)	
New sex partners in past 6 mo, no.			0.445
None	1335 (61.4)	2530 (62.4)	
1	555 (25.5)	976 (24.1)	
≥2	285 (13.1)	548 (13.5)	
Frequency of vaginal sex in past 6 mos.			0.625
<1 per week	724 (46.6)	1339 (48.1)	
1-2 per week	402 (25.9)	712 (25.6)	
>2 per week	427 (27.5)	735 (26.4)	
Condom use in vaginal sex in past 6 mos.			0.312
Always	447 (23.9)	723 (22.0)	
Frequently	433 (23.2)	749 (22.8)	
Sometimes	256 (13.7)	490 (14.9)	
Never	732 (39.2)	1323 (40.3)	
Condom use in anal sex in past 6 mos.			0.884
Always	235 (33.7)	391 (32.0)	
Frequently	121 (17.3)	211 (17.3)	
Sometimes	69 (9.9)	122 (10.0)	
Never	273 (39.1)	497 (40.7)	
Condom use in sexual intercourse in past 6 mos.			0.425
Always	376 (22.9)	644 (21.1)	
Frequently	362 (22.1)	657 (21.5)	
Sometimes	195 (11.9)	385 (12.6)	
Never	708 (43.1)	1364 (44.7)	
History of other STIs [c]			0.367
No	1783 (81.6)	3356 (82.5)	
Yes	402 (18.4)	711 (17.5)	

† p values were derived from Pearson chi-squared test statistics. Bolded p values denote statistical significance.
a. Light drinking was defined as <half a drink per day; moderate drinking as one half to 2 drinks per day; and heavy drinking as >2 drinks per day on average.
b. Circumcision status was assessed by study clinician.
c. Other STIs: Chlamydia, HSV, genital warts, Gonorrhea, Hepatitis B, Hepatitis C, non-gonococcal urethratitis, Syphilis

Table 4.2 Select participant characteristics between seronegative and seropositive men in Tampa, Cuernavaca and São Paulo.

Characteristics	Overall (N=2187)		MSW (N=1953)		MSM (N=234)	
	Seronegative	Seropositive	Seronegative	Seropositive	Seronegative	Seropositive
Overall	1918 (87.7)	269 (12.3)	1757 (90.0)	196 (10.0)	161 (68.8)	73 (31.2)
Age, *years*						
18-24	597 (31.1)	46 (17.1)	551 (31.4)	39 (19.9)	46 (28.6)	7 (9.6)
25-44	1057 (55.1)	183 (68.0)	957 (54.5)	125 (63.8)	100 (62.1)	58 (79.5)
45-70	264 (13.8)	40 (14.9)	249 (14.2)	32 (16.3)	15 (9.3)	8 (11.0)
p value[†]		**<0.001**		**0.004**		**0.006**
Country of residence						
Brazil	671 (35.0)	148 (55.0)	561 (31.9)	89 (45.4)	110 (68.3)	59 (80.8)
Mexico	682 (35.6)	53 (19.7)	655 (37.3)	44 (22.4)	27 (16.8)	9 (12.3)
US	565 (29.5)	68 (25.3)	541 (30.8)	63 (32.1)	24 (14.9)	5 (6.8)
p value[†]		**<0.001**		**<0.001**		0.113
Marital Status						
Single	853 (44.6)	122 (45.4)	752 (42.9)	74 (37.8)	101 (62.7)	48 (65.8)
Cohabiting	243 (12.7)	37 (13.8)	228 (13.0)	29 (14.8)	15 (9.3)	8 (11.0)
Married	663 (34.7)	81 (30.1)	630 (36.0)	72 (36.7)	33 (20.5)	9 (12.3)
Dvorced/Separated/Widowed	154 (8.1)	29 (10.8)	142 (8.1)	21 (10.7)	12 (7.5)	8 (11.0)
p value[†]		0.289		0.392		0.424
Education						
Less than high school	410 (21.4)	54 (20.1)	380 (21.7)	39 (19.9)	30 (18.6)	15 (20.5)
High school graduate	520 (27.2)	73 (27.1)	477 (27.2)	52 (26.5)	43 (26.7)	21 (28.8)
College or higher	982 (51.4)	142 (52.8)	894 (51.1)	105 (53.6)	88 (54.7)	37 (50.7)
p value[†]		0.860		0.772		0.851
Alcohol drinking[a]						
Light	612 (43.5)	80 (41.0)	566 (43.7)	51 (37.0)	46 (41.4)	29 (50.9)
Moderate	502 (35.7)	72 (36.9)	461 (35.6)	56 (40.6)	41 (36.9)	16 (28.1)

Characteristics	Overall (N=2187)		MSW (N=1953)		MSM (N=234)	
	Seronegative	Seropositive	Seronegative	Seropositive	Seronegative	Seropositive
Heavy	293 (20.8)	43 (22.1)	269 (20.8)	31 (22.5)	24 (21.6)	12 (21.1)
p value[†]		0.803		0.309		0.443
Cigarette smoking						
Never	409 (33.9)	46 (28.9)	383 (34.4)	29 (25.9)	26 (27.7)	17 (36.2)
Current	432 (35.8)	57 (35.8)	388 (34.9)	40 (35.7)	44 (46.8)	17 (36.2)
Former	366 (30.3)	56 (35.2)	342 (30.7)	43 (38.4)	24 (25.5)	13 (27.7)
p value[†]		0.345		0.128		0.445
Circumcision[b]						
No	1251 (65.2)	186 (69.1)	1127 (64.1)	125 (63.8)	124 (77.0)	61 (83.6)
Yes	667 (34.8)	83 (30.9)	630 (35.9)	71 (36.2)	37 (23.0)	12 (16.4)
p value[†]		0.217		0.938		0.300
Lifetime sex partners (either sex), *no.*						
None	104 (5.5)	17 (6.4)	100 (5.7)	14 (7.2)	4 (2.7)	3 (4.3)
1-3	495 (26.0)	36 (13.6)	474 (27.0)	31 (15.9)	21 (14.0)	5 (7.1)
4-7	407 (21.4)	47 (17.7)	386 (22.0)	38 (19.5)	21 (14.0)	9 (12.9)
8-18	459 (24.1)	67 (25.3)	420 (23.9)	51 (26.2)	39 (26.0)	16 (22.9)
≥19	440 (23.1)	98 (37.0)	375 (21.4)	61 (31.3)	65 (43.3)	37 (52.9)
p value[†]		**<0.001**		**0.001**		0.484
Lifetime female sex partners among MSW, *no.*						
None	--	--	2 (0.1)	0 (0)	--	--
1-3	--	--	478 (28.9)	32 (17.8)	--	--
4-17	--	--	789 (47.7)	87 (48.3)	--	--
≥18	--	--	385 (23.3)	61 (33.9)	--	--
p value[†]		--		**0.001**		--
Lifetime male anal sex partners, *no.*						
None	--	--	--	--	5 (3.4)	4 (6.1)
1-2	--	--	--	--	26 (17.7)	6 (9.1)

Characteristics	Overall (N=2187)		MSW (N=1953)		MSM (N=234)	
	Seronegative	Seropositive	Seronegative	Seropositive	Seronegative	Seropositive
3-10	--	--	--	--	70 (47.6)	26 (39.4)
≥11	--	--	--	--	46 (31.3)	30 (45.5)
p value†		--		--		0.100
New sex partners (either sex) in past 6mos, *no*						
None	1183 (61.9)	152 (57.4)	1122 (64.0)	126 (64.6)	61 (39.1)	26 (37.1)
1	492 (25.8)	63 (23.8)	454 (25.9)	43 (22.1)	38 (24.4)	20 (28.6)
≥2	235 (12.3)	50 (18.9)	178 (10.1)	26 (13.3)	57 (36.5)	24 (34.3)
p value†		**0.012**		0.256		0.798

Note. Bolded p values denote statistical significance at α=0.05. Cells that do not add up to 100 percent are due to missing values.
† P values were derived from chi-squared or fisher exact test statistics.
a. Light drinking was defined as <half drink per day; moderate drinking as half to 2 drinks per day; and heavy drinking as >2 drinks per day on average.
b. Circumcision status was assessed by study clinician.

Table 4.3 Incidence proportion of HPV 16 infection and 6-month persistent infection by study visit and baseline serostatus

A. Incident HPV 16 Infection

		Incident HPV 16 Infection						
	Visit	6 months	12 months	18 months	24 months	30 month	36 months	Total
	no. infections (%†)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	
Overall	Seronegative	64 (3.3)	48 (3.0)	34 (2.7)	22 (2.4)	10 (1.6)	9 (2.2)	187
	Seropositive	8 (3.0)	5 (2.1)	7 (3.8)	6 (4.3)	3 (2.9)	5 (6.7)	34
MSW	Seronegative	56 (3.2)	40 (2.8)	31 (2.7)	19 (2.3)	7 (1.2)	7 (1.9)	160
	Seropositive	6 (3.1)	3 (1.8)	6 (4.5)	2 (2.0)	1 (1.4)	4 (8.0)	22
MSM	Seronegative	8 (5.0)	8 (5.5)	3 (2.7)	3 (3.5)	3 (5.1)	2 (4.9)	27
	Seropositive	2 (2.7)	2 (3.0)	1 (2.0)	4 (10.5)	2 (6.7)	1 (4.0)	12

B. 6-month Persistent HPV 16 Infection

		6-month Persistent HPV 16 Infection					
	Visit	6 months	12 months	18 months	24 months	30 month	
	no. infections (%†)	n (%)	n (%)	n (%)	n (%)	n (%)	Total
Overall	Seronegative	20 (1.3)	18 (1.1)	14 (1.1)	6 (0.7)	5 (0.8)	63
	Seropositive	3 (1.3)	3 (1.3)	2 (1.1)	0 (0)	1 (1.0)	9
MSW	Seronegative	17 (1.2)	14 (1.0)	14 (1.2)	5 (0.6)	5 (0.9)	55
	Seropositive	3 (1.8)	2 (1.2)	1 (0.8)	0 (0)	1 (1.4)	7
MSM	Seronegative	3 (2.1)	4 (2.8)	0 (0)	1 (1.2)	0 (0)	8
	Seropositive	0 (0)	1 (1.5)	1 (2.0)	0 (0)	0 (0)	2

† % represents the percent of all participants at risk (men who were HPV negative at the prior visit and followed until the stated visit or later).

Table 4.4 Association of incident HPV 16 infection and 6-month persistent HPV 16 infection with baseline serostatus (seronegative vs. seropositive) among 2187 men in Tampa, Cuernavaca and São Paulo.

A. Incident HPV 16 infection

	Incident HPV 16 Infection (N=2187)					
	no. subjects	no. infections	HR	95% CI	Adjusted HR†	95% CI
HPV 16 Serostatus						
Negative	1918	187	1.00		1.00	
Positive	269	34	1.23	(0.85-1.77)	1.22	(0.83-1.79)

HR: Hazard Ratio. CI: Confidence interval.
†. Adjusted for age at enrollment, sexual practice (MSW and MSM) and lifetime number of sex partners.

B. 6-month persistent HPV 16 infection

	6-month Persistent Infection (N=1834)					
	no. subjects	no. infections	HR	95% CI	Adjusted HR†	95% CI
HPV 16 Serostatus						
Negative	1598	63	1.00		1.00	
Positive	236	9	0.96	(0.48-1.93)	1.05	(0.51-2.16)

HR: Hazard Ratio. CI: Confidence interval.
†. Adjusted for age at enrollment, sexual practice (MSW and MSM) and the number of new sex partners in past 6 months.

Table 4.5 Association of incident HPV 16 infection and 6-month persistent HPV 16 infection with baseline serostatus (seronegative vs. seropositive) for MSW and MSM in Tampa, Cuernavaca and São Paulo

A. Incident HPV 16 infection

	Incident HPV 16 Infection											
	MSW (N=1953)						MSM (N=234)					
	no. subjects	no. infections	HR	95% CI	Adjusted HR[a]	95% CI	no. subjects	no. infections	HR	95% CI	Adjusted HR[b]	95% CI
HPV 16 Serostatus												
Negative	1757	160	1.00		1.00		161	27	1.00		1.00	
Positive	196	22	1.18	(0.76-1.84)	1.60	(0.98-2.60)	73	12	0.91	(0.46-1.80)	1.23	(0.60-2.50)

HR: Hazard Ratio. CI: Confidence interval. MSW: Men who had sex with women. MSM: Men who had sex with men or men who had sex with men and women.
a. Adjusted for age at enrollment and lifetime number of sex partners. b. Adjusted for age at enrollment and circumcision.

B. 6-month persistent HPV 16 infection

	6-month Persistent HPV 16 Infection											
	MSW (N=1623)						MSM (N=211)					
	no. subjects	no. infections	HR	95% CI	Adjusted HR[a]	95% CI	no. subjects	no. infections	HR	95% CI	Adjusted HR[b]	95% CI
HPV 16 Serostatus												
Negative	1453	55	1.00		1.00		145	8	1.00		1.00	
Positive	170	7	1.09	(0.49-2.38)	1.05	(0.45-2.46)	66	2	0.53	(0.11-2.51)	1.03	(0.21-4.98)

HR: Hazard Ratio. CI: Confidence interval. MSW: Men who had sex with women. MSM: Men who had sex with men or men who had sex with men and women.
a. Adjusted for age at enrollment and the number of new female sex partners in past 6 months. b. Adjusted for age at enrollment.

Table 4.6 Association of incident HPV 16 infection and 6-month persistent HPV 16 infection with baseline serum antibody titer (continuous) among 2187 men in Tampa, Cuernavaca and São Paulo.

A. Incident HPV 16 infection

	Incident HPV 16 Infection											
	Overall (N=2187)				MSW (N=1953)				MSM (N=234)			
	Crude HR	95% CI	Adjusted HR[a]	95% CI	Crude HR	95% CI	Adjusted HR[b]	95% CI	Crude HR	95% CI	Adjusted HR[c]	95% CI
HPV 16 Antibody titer												
Per log(OD) increase	1.18	(0.99-1.41)	1.16	(0.97-1.39)	1.15	(0.93-1.43)	1.27	(0.99-1.61)	1.04	(0.76-1.43)	1.18	(0.85-1.64)

HR: Hazard Ratio. CI: Confidence interval. MSW: Men who had sex with women. MSM: Men who had sex with men or men who had sex with men and women.
a. Adjusted for age at enrollment, sexual practice (MSW and MSM) and lifetime number of sex partners.
b. Adjusted for age at enrollment, alcohol drinking and lifetime number of female sex partners.
c. Adjusted for age at enrollment and circumcision status.

B. 6-month persistent HPV 16 infection

	6-month Persistent HPV 16 Infection											
	Overall (N=1834)				MSW (N=1623)				MSM (N=211)			
	Crude HR	95% CI	Adjusted HR[a]	95% CI	Crude HR	95% CI	Adjusted HR[b]	95% CI	Crude HR	95% CI	Adjusted HR[c]	95% CI
HPV 16 Antibody titer												
Per log(OD) increase	1.05	(0.76-1.44)	1.10	(0.79-1.52)	1.06	(0.73-1.54)	1.06	(0.71-1.57)	0.92	(0.48-1.78)	1.20	(0.60-2.39)

HR: Hazard Ratio. CI: Confidence interval. MSW: Men who had sex with women. MSM: Men who had sex with men or men who had sex with men and women.
a. Adjusted for age at enrollment, sexual practice (MSW and MSM) and the number of new sex partners in past 6 months.
b. Adjusted for age at enrollment and the number of new sex partners in past 6 months.
c. Adjusted for age at enrollment.

Table 4.7 Association of incident HPV 16 infection and 6-month persistent HPV 16 infection with baseline serostatus (seronegative vs. seropositive) in the restricted analysis

A. Incident HPV 16 infection

	Incident HPV 16 Infection					
	Overall (N=1770)		MSW (N=1568)		MSM (N=202)	
	HR	95% CI	HR	95% CI	HR	95% CI
HPV 16 Serostatus						
Negative	1.00		1.00		1.00	
Positive	1.41	(0.92-2.15)	1.31	(0.77-2.22)	1.06	(0.49-2.29)

B. 6-month persistent HPV 16 infection

	6-month Persistent HPV 16 Infection					
	Overall (N=1811)		MSW (N=1603)		MSM (N=208)	
	HR	95% CI	HR	95% CI	HR	95% CI
HPV 16 Serostatus						
Negative	1.00		1.00		1.00	
Positive	0.93	(0.40-2.19)	0.90	(0.32-2.52)	0.85	(0.16-4.36)

CHAPTER 5:

CONCLUSIONS AND RECOMMENDATIONS

Data from our studies indicate that exposure to HPV 6, 11, 16 and 18, the four HPV types targeted in the currently license HPV vaccines, is common. Of 285 male residents of Tucson, Arizona, 28.8% of them were seropositive to HPV 16 and/or 18 at study entry. Similarly, approximately one third of 1477 participants of the multi-national male HPV natural history study were seropositive to at least one vaccine HPV type, with the percentage of 21.8% in U.S. site, 33.4% in Mexico site, and 49.1% in Brazil site. It is also noted that seroprevalence of individual vaccine HPV types is greatly elevated among men of different sexual practices. Seroprevalence of HPV 6, 11, 16 and/or 18 was twice as high among MSM and MSMW compared to MSW. Likewise, seroprevalence of individual HPV types was two fold or higher among MSW and MSMW.

Our findings suggest that the predominant predictors of seropositivity to HPV 6, 11, 16 and 18 are age and same-sex sexual behaviors. Seroprevalence increased significantly with age among young-to-middle-aged men, and declined among older men for HPV 6 and 11, whereas plateau for HPV 16, and continued to rise for HPV 18. MSM, compared to MSW, more likely to be seropositive to HPV 16 or 18. Similarly, men who practiced same-sex anal sex, compared to those who did not, were significantly more likely to be seropositive to HPV 6, 11, 16 and 18, respectively. The fluctuation of seroprevalence with age probably reflects age-related immune response and sexual behavior pattern over one's life span. The higher seroprevalence associated with the practice of same-sex anal sex is likely explained by the higher likelihood of simultaneous

HPV infections (oral, genital and anal) at multiple anatomic sites among MSM and MSMW. The finding may also imply that infection at mucosal epithelium is more likely to induce an immune response than infection at keratinized epithelium.

Also of the primary interest in this dissertation was the relationship between serum antibodies and subsequent risk of infection with the same HPV type or genetically related and un-related HPV types. Among 276 men free of HPV 16 at enrollment in Tucson, We did not detect statistically significant associations between the baseline serum antibodies to HPV 16 and/or 18 and subsequent risk of infection with homogeneous HPV types or related-HPV types. Of 2187 men residing in three countries who tested HPV 16 negative at enrollment, the risk of incident HPV 16 infection appeared to be lower in seropositive men compared to seronegative men within the first year of study period. The risk difference was more apparent among MSM and MSMW. So was the risk difference for 6-month persistent HPV 16 infection within the first year. However, data indicated that detection of HPV 16 serum antibodies at the baseline did not predict a man's risk of acquiring HPV 16 infection over time.

The findings of this dissertation support strategic vaccination of males as an effective preventive measure for HPV-related diseases and cancers in men and their sexual partners, men and women alike. Our data also provide important estimates of population exposure to vaccine HPV types for future studies modeling potential vaccine impact and vaccine cost effectiveness in men to provide further guidance for male vaccination policy.

Among many possible paths the future research may take, it is of primary interest for us to gain a better understanding of the natural history of humoral immune response to HPV infection. Prospective studies with repeated assessment of participant sexual behaviors, HPV DNA and serum antibody status and a multiple-year follow-up are needed to observe the lag time between the detection of type-specific incident HPV

infection and the detection of serum antibodies corresponding to the specific HPV type, to estimate type-specific seroconversion rates, to determine the longevity of type-specific serum antibodies in men, and to investigate factors associated with type-specific serum antibody development and persistence. Furthermore, prospective studies with similar design are also essential to explore potential influence of serum antibody presence on acquisition and clearance of infection with homogeneous HPV type and genetically related HPV types in men. The studies need to take into account various durations of serum antibody presence, and examine the associations between serum antibody detection and HPV acquisition and clearance by duration of antibody presence to determine if persistence of serum antibody plays a role in conferring protection.

As the choices made by individual studies of VLP-based immunoassay, control sera, and cutoff points could limit valid comparison of serum antibody measurements across studies, future studies focusing on establishing standard reference sera and determining correlates of commonly used VLP immunoassays could provide a comprehensive platform for interpretation of findings on serum antibody detection and associated factors across studies, and to advance our understanding of humoral immune response to HPV infection.

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APPENDICES

APPENDIX A: LITERATURE REVIEW

1. SEROEPIDEMIOLOGY OF HPV IN MEN

1.1. Introduction

Serological studies on HPV infection have been hampered by the difficulty of obtaining HPV virions from cell cultures to use as antigen targets for serological assay until recent years. This challenge was met through the invention of virus-like particle (VLP) technologies. VLPs are empty HPV viral capsids that contain no viral particles but appear morphologically identical to and contain the major neutralizing epitopes of the native HPV virion [1, 2]. Using recombinant DNA technology, the structural protein L1 or L2 in HPV viral capsid is expressed as virus-like particles (VLPs) in viral vectors such as vaccinia virus or baculovirus; the viral vectors are used to infect insect or mammalian cells to produce large quantities of VLPs, which formed the basis for VLP-based enzyme-linked immunosorbent assay (ELISA) for HPV antibody testing. Anti-HPV serum IgG antibodies detected by VLP-based assays are type-specific [3-5] and are most commonly used in HPV serology studies. The first L1 VLP-based assay that correlated with type-specific detection of HPV DNA was established in 1994 [6]. Since then, the use of VLP-based ELISA has led to our better understanding of serum antibody response to HPV infections.

1.2. Serum Antibody Response to Human Papillomavirus Infection

Serum antibodies elicited by natural HPV infection reflect cumulative exposures to HPV over time and across anatomic sites. The majority of type-specific seroconversion is observed within one year following incident HPV DNA detection, as evidenced in natural history studies of serum antibodies among women [7-10]. Andersson-Ellstrom and colleagues followed 98 adolescent girls aged 15-17 every 6

months for 2 years and observoed that of 6 girls who were initially seronegative and DNA negative, and who serocnverted during the follow-up, 5 tested positive for incident HPV 16 DNA at the visit prior to seroconversion [9]. Similarly, a median time of 8.3 months from detection of incident HPV 16 DNA to seroconversion was observed among 28 female university students who acquired HPV 16 during the follow-up in the study of Ho et al [8]. Carter et al reported that the median time to seroconversion for 56 women with incident HPV 6, 16 or 18 infection was 11.7, 11.8 and 12.6 months, respectively, among a cohort of 588 female university students aged 18-20 [7].

Anti-HPV serum antibodies appear to be stable over time and remain detectable even after a decade [10-13]. Not all individuals challenged by natural exposure to HPV develop antibody response detectable by current serology assays in the months following detection of infection [7, 8]. Carter et al observed that few seroconversions occurred after 18 months following incident HPV detection and the 18-month seroconversion rate was 68.8%, 59.5%, and 54.1% for incident HPV 6, 16, and 18, respectively [7]. A 12-month cumulative incidence of seroconversion of 56.7% (95% CI, 34.0–79.4) was reported by Ho et al [14]. Although issues like differential seroconversion measurable by VLP-based assay following DNA detection of different HPV types, and unknown longevity of anti-HPV serum antibodies could limit faithful translation, HPV serology remains a useful means for estimating cumulative HPV exposures in the population.

Limited number of studies have investigated prevalence of serum antibodies in men, with a majority of them evaluating HPV 16 seroprevalence. Seroprevalence of HPV 16 ranges from 5.1% to 47.5% depending on the study population examined, and choice of serological assay, control serum and cut-off point for determining seropositivity. Hagensee et al reported HPV 16 seroprevalence of 47.4% among 101 HIV-negative and

42.2% among 154 HIV-positive homosexual or bisexual men who participated in Seattle-King County Department of Public Health AIDS Prevention Project [15]. A capture ELISA was applied and a cut-off point determined using (1) similarly tested sera from a group of women without sex partners and evidence of HPV 16 DNA; and (2) a population-based maximum likelihood method. Using a similar assay and a cut-off point chosen based on similarly run sera from children <10 years of age with no evidence of HPV infection, Slavinsky et al observed a HPV 16 seroprevalence of 36.1% among 687 STI clinic attendees in New Orleans [16]; and Thompson and colleagues estimated a seroprevalence of 18.7% among 786 HIV-negative, heterosexual men attending 5 public STI clinics in the U.S. [17]. Using a direct ELISA assay, seropositivity to HPV 16 among 404 Jamaican male STI clinic attendees was 29% as determined by Strickler et al [18], and 9-32% among 219 Danish and 11-35% among 88 Greelandic male STI clinic patients in the study of Svare et al using various cut-off points [19].

Seroprevalence appeared to be consistently lower in population- and community-based studies than clinic-based studies. In general, the direct-binding ELISA assay showed higher sensitivity for serum antibody detection than the single-epitope competitive ELISA assay when applied in similar populations. HPV 16 seropositivity was found in 13.0% of 3110 male participants of National Health and Nutrition Examination Survey (NHANES 1991-94) in which a direct-binding ELISA assay and a cut-off value was chosen on the basis of optimal sensitivity and specificity [20]; whereas seropositivity was observed in 5.1% of 2128 NHANES 2003-2004 male participants using a multiplexed, competitive immunoassay and a predetermined cut-off value [21]. However, it was possible that besides the assay difference, other factors such as cohort effect might have contributed to the difference in seroprevalence between the two NHANES populations. Likewise, approximately 12% of 462 male residents aged 18-40 in two U.S.

cities were HPV 16 seropositive as determined by a capture ELISA assay [22] and a cut-off point estimated using similarly tested sera from children <10 years old with no prior history of warts. In comparison, using a direct ELISA assay and a similar method to determine the cut-off value, Kreimer et al detected a HPV 16 seroprevalence of 14.1% among 340 men aged 18-76 participating in an oral cancer screening program and an oral HPV prevalence study [23].

Seroprevalence of other oncogenic genital HPV types evaluated in men include HPV 18, 31, 33, 45, 52 and 58. Seroprevalence of HPV 18, 31, 33 and 45 were 11.1%, 7.4%, 13.0% and 11.1% among 54 male participants of an epidemiologic study in South Africa using a direct ELISA assay [24]. Seroprevalence were 18.8% and 9.7% for HPV 18 and 33 among 340 men in the study of Kreimer et al [23]. Using a competitive ELISA assay, Dunne et al and Markowitz et al reported a seroprevalence of 5.4% and 1.5% for HPV 18, respectively [21, 22].

Few studies have evaluated serum antibodies to HPV 6 or 11 detectable by VLP-based assays. The seroprevalence was 40.6% for HPV 6 among HIV-negative and 31.8% among HIV-positive homosexual and bisexual men [15]. Seroprevalence of HPV 6/11 was 31.6% among STI clinic attendees in the U.S. [16], and 9.7% among residents of two U.S. cities using capture ELISA [22]. In comparison, HPV 11 seroprevalence was 4.7% in NHANES 1991-1994 participants with a direct ELISA assay [25], and 2.0% in HANES 2003-2004 participants with a multiplexed, competitive assay [21].

1.3. Risk Factors Associated with Seroprevalence in Men

A growing body of literature has examined factors associated with HPV seroprevalence in women determined by VLP-based immunoassay, In contrast, little is known about factors associated with seroprevalence in men. Despite differences in study populations examined, choice of serological assays, control population and cut-off

points selected to determine seropositivity, evidence that emerges from the literature in men suggests that age, lifetime and recent number of sex partners and the practice of same-sex sexual intercourse are the most consistent predictors of HPV 16 seropositivity in men.

In a cross-sectional study that investigated factors associated with HPV 16 seropositivity among 219 HIV-negative Danish men and 88 Greenlandic men attending STI clinics in Copenhagen and Nuuk, risk of seropositivity was found to be significantly associated with increasing number of lifetime sex partners, a self-reported history of any STI, and sexual preference [19] in Denmark but not in Greenland. The risk of seropositivity was higher for men with ≥40 partners (OR, 2.8; 95% CI: 1.0–7.7) compared to men with 1–19 partners, for those with a self-reported history of any STI (OR, 2.6; 95% CI: 1.1-6.3), and for bisexual men compared with heterosexual men (OR, 3.0; 95% CI: 1.2-7.6). Strickler and colleagues demonstrated that older age ($p<0.05$), greater lifetime number of sexual partners ($p<0.01$) and longer years of sexual activity ($p<0.05$) were each significantly associated with seropositivity to HPV 16 among Jamaican STI clinic attendees [18]. In addition, sexual behavior in the past year including tears or bruises of the penis during sex ($p=0.01$), a greater number of sexual partners ($p=0.01$) and less regular use of condoms ($p=0.05$) were also independently associated with HPV 16 seroprevalence.

Hagensee et al observed that among HIV-seronegative men participating in an AIDS Prevention Project, having >50 lifetime male sex partners (OR, 3.8; 95% CI, 1.6-9.0) was significantly associated with detection of antibodies to HPV-16; and age >35 (OR, 2.0; 95% CI, 0.8-4.7) was suggestive of an independent association [15]. Slavinsky et al confirmed the association of HPV 16 serum antibody detection with age >30 (OR, 2.55; 95% CI: 1.50-4.40) and a history of STI (syphilis; OR, 1.60; 95% CI: 1.50-2.43)

among a cohort of STI clinic patients [16]; Whereas Thompson and colleagues observed in a similar group of men that Hispanic men compared to White men (OR, 2.0; 95% CI: 1.0–3.8]), men aged >20 compared to those aged ≤20 (OR, 3.4; 95% CI: 1.4–7.8), and men having had 1 occasional sex partner in preceding 3 months compared to those with none (OR, 1.7; 95% CI: 1.1–2.8) were more likely to test seropositive for HPV 16 [17].

While lower seroprevalence of HPV 16 was detected among nationally representative samples of men and community male residents than in the high-risk male populations, factors associated with detection of anti-HPV 16 serum antibodies in population-based and community-based studies resemble those detected in STI clinic-based studies. Data from the survey of NHANES 1991-1994 participants indicated that men who had first intercourse at age 18 or younger (OR, 2.5; 95% CI: 1.7–4.0), had a history of same-sex sexual intercourse (OR, 6.1; 95% CI: 2.7–14.0), or had ≥10 year of sexual activities (OR, 3.6; 95% CI: 1.8–7.3) were more likely to be HPV 16 seropositive [20]. Age ≥30 (OR, 1.9; 95% CI: 0.9–4.1) was suggestive, but was not statistically significant. In addition, there appeared to be an interaction between race and residence, with urban non-Hispanic White men (OR, 2.8; 95% CI: 1.6–5.0) and Black men (OR, 2.1; 95% CI: 1.2–3.5) at higher risk of HPV 16 seropositivity [20]. In comparison, age and lifetime number of sex partners were positively and significantly associated with seroprevalence of HPV 16 and/or 18 in the survey of NHANES 2003-2004 male participants [21]. Similar associations were also reported by Dunne et al among predominantly heterosexual men where older age (OR, 6.8; 95% CI: 3.7, 12.8), a greater number of female partners in the last 3 months (1 vs. 0 partner: OR, 4.5; 95% CI: 1.6-12.6 ; and >1 vs. 0 partner: OR, 5.3; 95% CI: 1.8-15.8) and current smoking (OR, 1.9; 95% CI: 1.1-3.2) were independently associated with higher seroprevalence of HPV 6, 11, 16 and/or 18 [22]. In addition, Kreimer et al confirmed that seroprevalence of HPV

16, 18 and/or 33 was significantly associated with ever having same-sex oral sex (OR, 2.9; 95% CI: 1.2–7.1) as well as participant HIV serostatus (OR, 2.1; 95% CI: 1.2–3.9) [23].

2. THE ROLE OF NATURAL IMMUNITY IN THE NATURAL HISTORY OF HPV INFECTION

2.1. Association of serum antibodies with subsequent risk of HPV acquisition

Few serology studies in women have assessed whether anti-HPV serum antibodies, induced by natural infection, were effective in protecting against subsequent infection with homologous or phylogenetically related HPV types. Findings from these studies have been inconsistent [26-28]. Viscidi et al investigated the risk of incident HPV 16 infection 5-7 years following detection of type-specific serum antibodies to HPV 16, 18 and 31 at enrollment among 7046 women enrolled in the Guanacaste Natural History Study using a direct ELISA assay, and failed to detect a significant difference in the risk of subsequent infection between baseline seropositive and seronegative women for any of these HPV types. Likewise, Trottier et al investigated the risk of incident HPV 16 infection by baseline serum antibody status among 1,902 women enrolled in the Ludwig-McGill cohort who were HPV 16 negative at baseline and followed every 4-6 months for up to 10 years using a similar assay [28]. The authors observed that the incidence rate was highest among women with the highest level of HPV-16 antibodies at baseline and there was no significant difference in incidence rates between women with the lowest antibody titers and those with the highest antibody titers (Lowest tertile 1.6 [95% CI: 1.3–2.1] vs. Highest tertile 2.1 [95% CI: 1.7–2.6]).

In contrast, Ho et al monitored 608 female college students with HPV16 antibody measured annually and HPV DNA measured every six months for a maximum of 3 years, and observed a protective effect of serum antibodies against incident infection

with HPV 16 (RR, 0.49; P=0.037) and its related types (P = 0.010) among young women who had persistent high antibody titer at ≥2 visits prior to the detection of infection [26]. Likewise, a protective effect was observed by Velicer et al among women aged 24-34 enrolled in the placebo arm of a HPV vaccine trial using cLIA, as evidenced by a lower incidence of HPV 6/11/16/18 infection and 6-month persistent infection among baseline seropositive women compared to seronegative women (incident infection: 1.0 [0.31–2.23] vs. 5.7 [4.68–6.84] per 100 woman-years; 6-month persistent infection: 0.4 [0.05–1.37] vs. 2.5 [1.89–3.35] per 100 woman-years) [29]

2.2. Gender Difference in Seroprevalence

It has been noted in a number of HPV serology studies that men and women appear to respond to HPV infection differently. Despite the higher DNA prevalence and risky sexual behaviors observed in men, consistently lower antibody titers and a lower seroprevalence were observed in men compared to their female counterparts [17, 19-21, 23, 30]. The gap in HPV 16 seroprevalence between men and women ranges from 10-40%. In spite of variation in assays used, a 10% difference was observed in both studies of Stone et al [20] and Markowitz et al [21] among NHANES participants. The difference was 12% among STI clinic attendees in 5 U.S. cities [17] and 28% among oral cancer screening program and oral HPV study participants in Baltimore, U.S. [23]. In contrast, an up to 40% difference was detected among STI clinic patients in Denmark and Greenland [19]. These gender-related differences are likely an indication of differential immune responses induced by infection at keratinized epithelium versus that at mucosal epithelium.

To date, no study has evaluated the role of serum antibodies in prevention of subsequent HPV infection in men. This dissertation will be the first to explore potential

associations and to shed lights on the role of natural immunity in the natural history of HPV infection in men.

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