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Seroprevalence of Human Papillomavirus (HPV) Type 6 and 16 Vary by Anatomic Site of HPV Infection in Men

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Abstract

Background—It is largely unknown if anti-HPV serum antibody responses vary by anatomic site of infection in men.

Methods—This study assessed type-specific anti-HPV serum antibody prevalence associated with corresponding HPV DNA detection in the external genitalia and the anal canal of 1587 heterosexual men and 199 men who have sex with men (MSM).

Results—We observed that HPV 6 and 16 seroprevalence was higher in the presence of same HPV type infection in the anal canal compared to the presence in the external genitalia only, and among MSM compared to heterosexual men. Seropositivity to HPV 6 was strongly associated with HPV 6 DNA detection in the anal canal but not in the external genitalia alone among both heterosexual men (Adjusted Prevalence Ratio (APR), anal+/genital+ vs. anal-/genital-: 4.2 [95% CI: 1.7-10.5]; anal+/genital- vs. anal-/genital-: 7.9 [95% CI: 3.7-17.0]) and MSM (APR, anal+/genital+ vs. anal-/genital-: 5.6 [95% CI: 2.7-11.9]; anal+/genital- vs. anal-/genital-: 3.2 [95% CI: 2.1-4.9]). Similar associations between seropositivity to HPV 16 and anal HPV 16 DNA detection were only observed in MSM (anal+/genital+ vs. anal-/genital-: 3.1 [95% CI: 2.0-5.0]; anal+/genital- vs. anal-/genital-: 2.2 [95% CI: 1.3-3.5]).

Conclusion—Our data demonstrated that seroprevalence varied by anatomic site of HPV infection, suggesting differences in epithelium type present at these anatomic sites may be relevant.

Impact—Our finding is instrumental in advancing our understanding of immune mechanism involved in anatomic site-specific antibody response.

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Keywords

Human Papillomavirus (HPV); heterosexual men; men who have sex with men (MSM); seroprevalence; external genitalia; anal canal

INTRODUCTION

Human Papillomavirus (HPV) infection has been established as the cause of cervical, anal, penile, vaginal and vulvar cancers, and a subset of head and neck cancers (1). Detection of HPV infection at multiple anatomic sites is not uncommon in healthy adults and has been reported in a number of large epidemiologic studies (2, 3). A fundamental question is whether serum antibody responses vary by anatomic site of HPV infection. Answers will further our understanding of differences in population seroprevalence related to gender and sexual orientation. Consistent gender gaps in population seroprevalence have been well documented (4). Studies have also shown that men who practice same-sex intercourse have higher seroprevalence of vaccine HPV types (HPV 6, 11, 16, and 18) than do those with heterosexual relationship, suggesting differences in anatomic site infected with HPV may be relevant (5-7). The present study assesses type-specific vaccine HPV seroprevalence in association with corresponding HPV DNA detection at two different anatomic sites in men, the external genitalia and the anal canal, to determine whether anti-HPV serum antibody status varies by anatomic site of infection. Due to the sparse data for HPV 11 and 18, the current report only includes data for HPV 6 and 16.

METHODS

Study Population

We analyzed enrollment data from the *HIM Study*, an ongoing multi-national HPV natural history study in men conducted in Tampa, United States, São Paulo, Brazil, and Cuernavaca, Mexico. Details of the study cohort have been described elsewhere (8). In brief, healthy men were recruited using the following inclusion criteria: ages 18–70 years; residents of one of the 3 study sites; no prior diagnosis of penile and anal cancers or genital warts; no symptoms of or current treatment for sexually transmitted infections (STIs) including HIV/AIDS; no concurrent participation in an HPV vaccine study; and no history of imprisonment, homelessness or drug treatment in the past 6 months. At enrollment participants were administered a computer-assisted self-interview of sexual and medical history, and a clinical examination of the anogenital area. Exfoliated cell samples of the external genitalia and 10ml venous blood were collected. Collection of exfoliated cell samples from the anal canal was optional and only obtained from men who consented to the procedure.

A total of 4074 men were enrolled in the HIM Study between June 2005 and September 2009. Funding availability permitted PCR and genotyping of anal samples collected from the first 1971 participants who agreed to the optional anal sampling. Of these, 1786 heterosexual men and MSM who also had survey information, serology and genital HPV DNA results available from the enrollment visit were included in the current analysis. To assess potential selection bias stemming from optional anal sampling we compared characteristics of men included in the current analysis with those in the full HIM study cohort. We observed no differences in terms of demographics, life styles or sexual behaviors in the current study cohort, although the current participants were slightly older ($p=0.002$) and more likely to report one or no recent female sex partners ($p=0.002$) (Supplementary data).

HPV Serum Antibody Testing

Testing of anti-HPV serum antibodies was performed using virus-like particle (VLP)-based enzyme-linked, immunosorbent assays (ELISA) (9). 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 expressed in optical density (OD). The mean and standard deviation (SD) of absorbance values were estimated based on seroreactivity of sera from children ages 0-10 years. Five standard deviations above the mean absorbance value was used as the cut point to determine 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 swabs were used to collect exfoliated skin cells from the penis (coronal sulcus; glans; and ventral and dorsal areas of shaft) and scrotum, and later combined to form a single specimen. Using a new swab, exfoliated cells were collected from between the anal os and the dentate line and placed into standard transport medium as a separate specimen. All specimens were stored at -80°C until use. DNA was extracted from exfoliated skin cell samples using the QIAamp DNA Mini Kit (QIAGEN, Valencia, CA) and tested for HPV DNA using PGMY 09/11 consensus primer system. The Linear Array Genotyping Protocol (Roche Diagnostics, Indianapolis, IN) was applied to detect 37 genital HPV types. Presence of human β -globin was tested to assure sample adequacy and was detected in both genital and anal HPV DNA samples in 82% of 1786 men.

Statistical Analysis

Participants were classified as men who had sex with women (MSW, n=1587, 88.9%), men who had sex with men (MSM, n=99, 5.5%), and men who had sex with men and women (MSMW, n=100, 5.6%) based on their responses to multiple baseline survey questions regarding their recent and lifetime sexual behaviors. Given the small number of MSM and MSMW, and their shared practice of same-sex sexual behaviors, MSM and MSMW were combined into the group "MSM" in the current analysis. Four categories of anogenital HPV DNA status were defined on a type-specific basis: HPV positive at both the anal canal and external genitalia (anal+/genital+), positive at the anal canal only (anal+/genital-), positive at the external genitalia only (anal-/genital+), and HPV negative at both sites (anal-/genital-). Serum antibody levels corresponding to site-specific anogenital infections were compared on a pairwise manner using Wilcoxon rank-sums test. The 95 percent confidence intervals (95% CI) of seroprevalence were estimated following a binomial distribution. A modified Poisson regression approach was used to estimate the associations between seroprevalence and anogenital HPV DNA status, measured by prevalence ratio (PR) and its 95% CIs (10). Covariates considered for adjustment were age, country of residence, marital status, educational attainment, alcohol drinking, smoking, circumcision, the number of recent and lifetime sex partners, frequency of sexual intercourse, condom use, self-reported history of other STIs and HSV-2 serostatus. Individual factors that were statistically significant at the level of 0.1 along with anogenital HPV DNA status were considered for inclusion in the multivariable models. A backward elimination procedure was used for covariate selection.

RESULTS

The majority of MSW and MSM was 25-44 years of age (56.5% vs. 65.8%), college-educated (54.9% vs. 56.3%), never-smokers (56.3% vs. 58.3%), light-to-moderate drinkers (2 drinks per day: 61.1% vs. 56.3%), uncircumcised (61.1% vs. 71.4%), and had one or more recent sex partners (77.1% vs. 66.8%), and three or more lifetime sex partners (69.8% vs. 74.9%).

Anti-HPV 6 and 16 serum antibody levels were significantly higher in men with corresponding anal HPV infection (anal+/genital+ or anal+/genital-) than those with genital HPV infection alone (anal-/genital+) among MSM (Figure 1). No differences were observed among MSW. Among all anal HPV positive men (anal+/genital+ or anal+/genital-), anti-HPV 6 serum antibody levels were significantly higher in MSM than in MSW (anal+/genital+: $p=0.0430$; anal+/genital-: $p=0.0376$), whereas anti-HPV 16 serum antibody levels were higher in MSM than in MSW among men with anal HPV infection alone (anal+/genital-: $p=0.0003$). No difference in serum antibody levels between MSM and MSW was observed among men with genital HPV infection alone.

The association of HPV seropositivity with anogenital HPV DNA detection was determined on a type-specific basis (Table 1). For HPV 6, MSW with an anal HPV infection, regardless of the presence of a genital co-infection, were more likely to be seropositive than were MSW who tested HPV DNA negative at both sites (Adjusted PR, APR, anal+/genital+: 4.2 [95% CI: 1.7-10.5]; anal+/genital-: 7.9 [95% CI: 3.7-17.0]). HPV 6 seropositivity was not associated with detection of genital HPV 6 DNA alone in MSW. Similarly, in MSM HPV 6 seropositivity was strongly associated with anal HPV 6 DNA detection (APR, anal+/genital+: 5.6 [95% CI: 2.7-11.9]; anal+/genital-: 3.2 [95% CI: 2.1-4.9]) but not with genital HPV 6 DNA detection alone. For HPV 16, detection of HPV DNA at either anogenital site or both sites was not associated with seropositivity in MSW. In contrast, presence of HPV 16 DNA at the anus was associated with HPV 16 seropositivity in MSM (APR, anal+/genital+: 3.1 [95% CI: 2.0-5.0]; anal+/genital-: 2.2 [95% CI: 1.3-3.5]).

DISCUSSION

To our knowledge, this study is the first to examine the likelihood of seropositivity in association with HPV DNA detection at multiple anatomic sites in men. Our data show that serum antibody levels and seroprevalence of HPV 6 and 16 were consistently higher in men with corresponding anal HPV infection, regardless of genital co-infection, compared to men with genital HPV infection alone. In addition, seroprevalence was higher in MSM than in heterosexual men who had same HPV DNA detected at the same anatomic site. Furthermore, we showed that HPV 6 seropositivity was strongly associated with concurrent detection of anal HPV 6 DNA, but not concurrent detection of genital HPV 6 DNA alone, in both MSW and MSM. Independent associations between HPV 16 seropositivity and anal HPV 16 DNA detection were only observed in MSM.

Our finding is consistent with previous observations that HPV seroprevalence was not associated with concurrent detection of genital HPV DNA in healthy adults (5, 11, 12). Given the lower incidence of HPV 6 infection in the anus compared to the external genitalia (1.2 vs. 3.6 per 1000 person-months), and relatively comparable clearance at both sites (64.7% at 6-month visit vs. 50% at 6.4 months) previously reported for the *HIM Study* participants (8, 13), higher HPV 6 seroprevalence in men with anal HPV 6 infection compared to those with genital HPV 6 infection alone observed in the current study is unlikely caused by differences in acquisition and clearance of anal and genital HPV. The differential seroprevalence linked to anatomic site-specific HPV DNA detection in men may be explained by the type of epithelium present at each anatomic site. Antigen presentation to the immune system at a mucosal epithelium (e.g. anus, cervix), compared to that at a keratinized epithelium (e.g. shaft, glans in circumcised men), may provide more direct access to the lymphatics and draining lymph nodes where immune responses are initiated, resulting in earlier and stronger antibody responses (14). Furthermore, the histology of the anal canal closely resembles that of the cervix with a transformation zone (15). The similarity in anatomy of the cervix and the anus suggests that divergent seroprevalence

observed in genital and anal HPV positive men likely mirrors gender-related differences in seroprevalence observed in population-based studies.

A higher HPV 6 and 16 seroprevalence was observed in MSM than in MSW for every category of anogenital infection, particularly in men with anal HPV infection. Recent data from the *HIM Study* suggest that a greater proportion of MSM than MSW who tested positive for anal HPV at baseline exhibited 6 month persistence (72.8% vs. 0% for HPV 16; 53.3% vs. 21.1% for HPV 6) (13). It is likely that the prolonged anal HPV infection harbored by MSM may have contributed to the higher seroprevalence observed in anal HPV-positive MSM compared to anal HPV-positive MSW. It is also likely that repeated anal exposures to HPV among previously infected MSM results in anamnestic responses, giving rise to the elevated seroprevalence in MSM. In addition, it is possible that direct sexual contact with an infected male partner during receptive anal intercourse allows viral transmission to the squamocolumnar junction of the anal canal where there is little keratinization, resulting in more efficient viral antigen detection by the immune system and stronger antibody responses. In contrast, anal HPV infection detected in MSW, in the absence of receptive anal sex, is likely acquired via auto-inoculation or inoculation through indirect contacts with infected female partners (16-18), possibly at the lowest part of the anal canal where the tissue is markedly keratinized, making it less accessible for immune recognition.

A major limitation of the present study is that HPV serostatus and DNA status was simultaneously assessed. Hence the temporal relationship between anatomic site-specific infection and seroreactivity could not be established. Associations detected between HPV DNA status and serostatus were subject to measurement errors due to the unknown duration of HPV DNA and serum antibody detectability, time lags in serum antibody development, limited seroconversion rates and waning of antibody responses over time, and therefore, may not represent the true association between incident anogenital HPV detection and subsequent serum antibody development.

In summary, in the current study HPV 6 and 16 serum antibody status varies by anogenital site infected with HPV and by sexual orientation. In addition, seroprevalence of HPV 6 and 16 is positively associated with the detection of corresponding HPV DNA in the anal canal but not in the external genitalia alone. Our data suggest that the involvement of different epithelia may underlie these observations. Prospective studies that assess serum antibody development following HPV detection at various anatomic sites are needed to further our understanding of anatomic-site specific serum antibody responses.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Serum antibody levels (Median, inter-quartile range, minimum and maximum) corresponding to anogenital site-specific HPV detection among HPV positive men.

Table 1

Likelihood of HPV 6 and 16 seropositivity associated with anogenital HPV DNA status.

	MSW (N=1587)				MSM (N=199)			
	n	Seroprevalence (95% CI)	PR (95% CI)	Adjusted PR (95% CI)	n	Seroprevalence (95% CI)	PR (95% CI)	Adjusted PR (95% CI)
HPV 6 DNA status								
anal -/ genital -	1484	6.0 (4.8-7.3)	1.00		170	26.5 (20.0-33.8)	1.00	1.00
anal +/- genital +	11	27.3 (6.0-61.0)	4.5 (1.7-12.2) ^a	4.2 (1.7-10.5) [*]	3	100 (29.2-100.0)	3.8 (2.9-4.9) ^b	5.6 (2.7-11.9) [*]
anal +/- genital -	10	40.0 (12.2-73.8)	6.7 (3.0-14.6) ^a	7.9 (3.7-17.0) [*]	14	85.7 (57.2-98.2)	3.2 (2.3-4.5) ^b	3.2 (2.1-4.9) [*]
anal -/ genital +	82	11.0 (5.1-19.8)	1.8 (0.96-3.5) ^a	1.9 (0.98-3.5)	12	16.7 (2.1-48.4)	0.6 (0.2-2.3) ^b	0.5 (0.1-2.1)
HPV 16 DNA status								
anal -/ genital -	1455	10.9 (9.3-12.6)	1.00		171	31.6 (24.7-39.1)	1.00	1.00
anal +/- genital +	8	25.0 (3.2-65.1)	2.3 (0.7-7.7) ^c	2.5 (0.7-8.3)	5	80.0 (28.4-99.5)	2.5 (1.6-4.1) ^d	3.1 (2.0-5.0) [*]
anal +/- genital -	20	15.0 (3.2-37.9)	1.4 (0.5-4.0) ^c	1.4 (0.5-3.7)	11	63.6 (30.8-89.1)	2.0 (1.2-3.3) ^d	2.2 (1.3-3.5) [*]
anal -/ genital +	104	15.4 (9.1-23.8)	1.4 (0.9-2.3) ^c	1.4 (0.9-2.3)	12	25.0 (5.5-57.2)	0.8 (0.3-2.2) ^d	0.2 (0.05-1.3)

Note. PR: Prevalence ratio. CI: Confidence interval.

^{*} denote statistically significant associations (p<0.05).^aThe final model adjusted for country and recent number of female sex partners^bThe final model adjusted for country, education and lifetime number of male anal sex partners.^cThe final model adjusted for age and HSV-2 serostatus.^dThe final model adjusted for age and lifetime number of male anal sex partners.