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The Natural History of Human Papillomavirus Related Condyloma

In a Multinational Cohort of Men

by

Gabriella M. Anic

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
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ABSTRACT

Human papillomavirus (HPV) is the most common sexually transmitted infection in the United States, but few studies have examined the progression from HPV infection to disease in men. Genital condyloma are the most common clinical manifestation of HPV infection. Though not associated with mortality, condyloma are a source of emotional distress, and treatment is often painful with a high recurrence rate. The aims of this study were to examine the distribution of HPV types present on the surface of condyloma, estimate the incidence of condyloma overall and after type-specific HPV infections, assess the sociodemographic and sexual behavior factors independently associated with incident condyloma, and examine the concordance between HPV types detected on the surface and in the tissue of condyloma. Participants included 2,487 men from the United States, Brazil, and Mexico who were enrolled in the prospective HPV in Men (HIM) Study and followed every six months for up to four years. At each study visit men completed a computer-assisted-self-administered risk factor questionnaire and samples of healthy penile skin were obtained to test for HPV DNA. A trained clinician examined men for the presence of condyloma and swabbed the surface of lesions to test for HPV DNA. Men were followed for a median of 17.9 months and 112 incident condyloma were identified. Thirty-four external genital lesions were also biopsied to test for HPV within the lesion tissue. PCR was used to test for HPV DNA and Linear Array was used to genotype 13 oncogenic and 24 non-oncogenic HPV types in samples obtained from swabbing the lesion surface. The LiPa assay was used to genotype 20 HPV types in biopsy samples. The Kaplan-Meier method was used to estimate incidence and Cox proportional hazards models were used to examine factors

independently associated with incident condyloma. Using biopsy samples as the gold standard, sensitivity and specificity were calculated to examine concordance between HPV types detected on the surface and within the tissue of condyloma. Condyloma incidence was 2.35 per 1,000 person-years. HPV 6 (43.8%), 11 (10.7%), and 16 (9.8%) were the most common types detected on condyloma. The probability of developing condyloma within 24-months of an incident HPV 6/11 infection was 14.6% (95% confidence interval (CI): 7.5-21.1). The median time to condyloma development was 17.1 months (95% CI: 12.4-19.3), with the shortest time to detection observed among men with incident HPV infections with types 6/11 only (6.2 months; 95% CI: 5.6-24.2). Factors associated with condyloma were incident HPV 6/11 infection (hazard ratio (HR)=12.42; 95% CI: 3.78-40.77), younger age (HR=0.43; 95% CI: 0.26-0.77; 45-70 vs. 18-30 years), high lifetime number of female partners (HR=5.69; 95% CI: 1.80-17.97); ≥ 21 vs. 0), and sexual behaviors in the previous three months including infrequent condom use (HR=2.44; 95% CI: 1.16-5.14; <half the time vs. always), number of male sexual partners (HR=4.53; 95% CI: 1.68-12.20; ≥ 3 vs. none), frequent vaginal intercourse (HR=4.14; 95% CI: 1.32-13.01); ≥ 21 times vs. none), having a partner with condyloma (HR=2.38; 95% CI: 1.01-5.61), and being diagnosed with a sexually transmitted infection (HR=1.99; 95% CI: 1.17-3.39). HPV 6/11 plays an important role in condyloma development with the highest incidence and shortest time to condyloma development observed among men with incident HPV 6/11 infections. Recent sexual history was also strongly associated with incident condyloma in men, suggesting that prevention efforts targeting behavioral modification may be effective at reducing condyloma incidence among men who are not vaccinated. Samples obtained from the surface of condyloma lesions were both sensitive and specific as markers for the presence of any HPV, HPV6 and HPV11 in condyloma tissue, suggesting that sampling

the surface of condyloma is a non-invasive and accurate marker of the HPV types present within the tissue.

CHAPTER 1: INTRODUCTION

STUDY PURPOSE

Human papillomavirus (HPV) is the most common sexually transmitted infection in the United States (US), with an estimated 6.2 million new cases each year [1]. HPV is an established cause of cervical cancer and is also associated with cancers of the oropharynx, anus, penis, vulva and vagina [2]. Over 100 HPV types have been identified and approximately 40 of these types infect the anogenital region. HPV types are classified as oncogenic types (e.g. 16, 18, 31, and 45) that are associated with intraepithelial neoplasia and invasive carcinoma, and non-oncogenic types (e.g. 6 and 11) that are associated with benign conditions such as condyloma.

Estimates of the prevalence of HPV in men has been as high as 73%. Recent studies of men in the US have reported that approximately 50% of men are positive for at least one known HPV type, and an additional 10-15% are positive for one or more unknown HPV types [3-5]. The probability of acquiring a new HPV infection over a 12-month period is 29-39% [3, 5, 6]. Incidence of HPV infection is not associated with age in men and remains consistent across the lifespan [3].

The majority of HPV infections are asymptomatic with an estimated 70% of incident infections clearing within one year [7]. Persistent infections can progress to disease and anogenital condyloma is the most common clinical manifestation of HPV infection [8]. Though condyloma are not associated with mortality, they are a source of physical discomfort, emotional distress and reduced quality of life [9, 10]. Condyloma have a high transmission rate between sexual partners; approximately 65% of individuals who have a sexual partner with condyloma will develop condyloma

themselves [11]. Treatment is often ineffective with about one-fourth of cases recurring within 3 months of treatment [12]. The high recurrence rate is associated with high medical costs; approximately \$200 million is spent annually in the US to treat condyloma [13].

The quadrivalent vaccine Gardasil, which protects against HPV types 6, 11, 16, and 18, was approved in October 2009 by the U.S. Food and Drug Administration for use in males ages 9 to 26. Clinical trials have shown the vaccine to be effective at reducing the incidence of HPV infection and condyloma in men ages 16-26 [14]. Incidence rates of condyloma and estimates of time from HPV infection to condyloma detection are necessary parameters for modeling the effectiveness of prevention through vaccination.

To date, most research on the progression of HPV infection to disease has focused on women. Little is known about the natural history of HPV related disease in men, including incidence of condyloma, the prevalence of HPV types within condyloma, the proportion of type-specific HPV infections that progress to condyloma, and the time from an HPV infection to development of condyloma. Since male genital lesions are reservoirs for HPV infection, understanding the natural history of HPV related genital disease in men has the potential to not only reduce the burden of male disease, but also reduce the rate of HPV transmission to women.

Current incidence rates for condyloma among US men are based on data from private insurance claims [15-17]. These data likely underestimate true incidence since they exclude individuals who do not seek treatment or who are not privately insured. Likewise, little is known regarding the median time from HPV infection to condyloma detection with only one published study to date conducted among young university students positive for HPV 6/11 [18]. Likewise, only a few studies have examined sexual behavioral factors associated with the development of condyloma in men [19-22], and

most of these studies were among highly selective populations including STI clinic attendees [20, 21] and men who have sex with men [22]. Similarly, many studies examining risk factors for condyloma in women have also been in select populations such as university students [23], STI clinic attendees [20], and young women in the placebo arm of an HPV vaccine trial [24]. Given that treatment of condyloma is often ineffective, it is important to identify modifiable behavioral factors for prevention efforts, especially among individuals who do not receive vaccination.

Sampling methods can influence prevalence estimates of HPV types present in condyloma. Current standard practice is to diagnose condyloma by visual inspection, and biopsy samples are not often obtained to confirm the diagnosis [25]. As a result, studies that include individuals from a standard clinic setting sample the surface of condyloma lesions to estimate the prevalence of HPV genotypes in the lesion tissue [26-28]. It is possible that the HPV types detected on the surface of lesions may not represent the types present in the lesions themselves. Assessing whether sampling the surface of a condyloma lesion provides an accurate measure of the HPV types present within the tissue of the condyloma could provide support as to whether it is necessary to biopsy a condyloma to accurately estimate the prevalence of HPV types in the lesion.

SPECIFIC AIMS

The goal of this research is to examine the progression of HPV infection to condyloma development in men. The proposed research will be based on a sub-cohort of 2,487 men enrolled in the HPV in Men (HIM) Study. The HIM Study is a prospective study that examines the natural history of anogenital HPV infection in men ages 18-70 from the US, Brazil, and Mexico. The specific aims of this research are:

1. Estimate the incidence of condyloma, describe the prevalence of HPV types detected in incident condyloma and measure the time from type specific incident HPV infections to condyloma detection.
2. Identify sociodemographic and sexual behavioral factors associated with the incidence of condyloma.
3. Examine the concordance between HPV types detected on the surface and in the tissue of histologically confirmed anogenital condyloma.

CHAPTER 2: FIRST MANUSCRIPT: INCIDENCE AND HPV TYPE DISTRIBUTION OF CONDYLOMA IN A MULTINATIONAL COHORT OF MEN: THE HIM STUDY

ABSTRACT

Background: Data on the natural history of human papillomavirus (HPV) infection progression to genital warts (GW) in men are sparse. We described the distribution of HPV types in incident GW and estimated GW incidence and time from type-specific incident HPV infections to GW detection in a multinational cohort of men ages 18-70.

Methods: Participants included 2,487 men examined every 6 months and followed for a median of 17.9 months. Samples obtained from 112 incident GW were tested for HPV DNA by PCR. Genotyping tested for the presence of 37 HPV types.

Findings: Incidence of GW was 2.35 per 1,000 person-years with the highest incidence rate observed among men ages 18-30 (3.43 per 1,000 person-years). HPV 6 (43.8%), 11 (10.7%), and 16 (9.8%) were the most common types detected in GW. The 24 month cumulative incidence of GW among men with incident HPV 6/11 infections was 14.6% (95% CI: 7.5-21.1). The median time to any GW detection was 17.1 months (95% CI: 12.4-19.3), with the shortest time to detection observed among men with incident infections with HPV 6/11 only (6.2 months; 95% CI: 5.6-24.2).

Interpretation: HPV 6/11 plays an important role in GW development with the highest incidence and shortest time to GW development observed among men with incident HPV 6/11 infections.

Funding: National Cancer Institute.

INTRODUCTION

Anogenital human papillomavirus (HPV) is the most common sexually transmitted infection in the United State (US) [1]. Over 100 HPV types have been identified and approximately 40 of these infect the anogenital region. Genital warts (GW) are a common HPV related disease associated with HPV types 6 and 11 [12]. In the US, 5.6% of sexually active adults ages 18-59 have self-reported ever being diagnosed with GW [29] and 1% of US adults ages 18-45 are estimated to have GW at any given time [30]. Though GW are benign and not associated with mortality, they are a source of psychosocial distress [9] and can cause physical discomfort including pain, bleeding and itching [25]. GW are highly infectious; 65% of people who have sex with a partner with GW will develop GW themselves [11]. A high rate of recurrence makes treatment difficult and costly [31]. Approximately \$200 million is spent annually in the US for GW treatment [13].

HPV vaccination may be an effective approach for primary prevention of GW [32]. However, incidence rates for GW and estimates of time from HPV infection to GW detection are necessary parameters for modeling the effectiveness of GW prevention through vaccination. Few published studies have reported the HPV type distribution in GW [26-28, 33], the incidence of GW [15-17], and the time from HPV infection to GW in men [18]. Most published incidence rates of GW for US men are based on data from private insurance claims [15-17]. These data likely underestimate true incidence since they exclude individuals who do not seek treatment or who are not privately insured. Likewise, little is known regarding the median time from HPV infection to GW detection with only one published study to date conducted among young university students positive for HPV 6/11 [34].

The purpose of this study was to describe the prevalence of HPV types detected in newly acquired GW and estimate GW incidence and time from type specific incident HPV infections to GW detection in a multinational cohort of men ages 18-70.

METHODS

Study population

The HPV in Men (HIM) Study is a multinational prospective study of men ages 18-70 that examines the natural history of HPV infection in men. Participants were enrolled into the *HIM Study* between July 2005 and September 2009 and met the following inclusion criteria: (a) 18-70 years old; (b) resided in Southern Florida, US, Sao Paulo, Brazil or the state of Morelos, Mexico; (c) reported no previous diagnosis of penile or anal cancer; (d) reported no prior diagnosis of genital or anal warts; (e) had not participated in an HPV vaccine clinical trial; (f) reported no prior diagnosis of HIV or AIDS; (g) were not currently being treated for an STI; (h) had not been imprisoned, homeless or in drug treatment in the previous 6 months; and (i) were willing to complete 10 scheduled visits every six months over four years.

In the United States, men were recruited from a large university and the general population in Tampa, FL via flyers, brochures and advertisements in local and university newspapers. In Brazil, men were recruited from the general population of the metropolitan area of São Paulo through several advertisements and from a large urogenital care clinic. Participants in Brazil also included the partners of healthy women who had participated in an HPV natural history study in Sao Paulo. In Cuernavaca, Mexico, men were recruited through a state health plan, from local factories, and from the military. All participants provided written informed consent and study protocols were approved by Institutional Review Boards at each study site. A more detailed description of the study design and population has been reported previously [3, 4, 35]. The present

study includes 2,487 men who enrolled in the *HIM Study* before January 1, 2009, did not have GW detected at enrollment, and completed at least one 6-month follow-up visit.

Genital wart identification

GW were identified by visual inspection of the external genitalia by a trained clinician at each clinic visit. All GW were sampled with a saline pre-wetted Dacron swab for the presence of HPV DNA. If multiple GW were detected, a separate specimen was obtained from each lesion. Specimens were also obtained from healthy genital skin on the coronal sulcus/glans penis, penile shaft, and scrotum for HPV DNA testing. GW were sampled before healthy genital skin to avoid inter-specimen contamination. Lesions that appeared to be related to Herpes Simplex Virus or a benign condition such as skin tags or cysts were not sampled for HPV DNA.

HPV DNA testing

DNA was extracted from samples using the QIAamp Mini kit following the manufacturer's instructions (Qiagen, Valencia, CA). The polymerase chain reaction (PCR) consensus primer system PGMY 09/11 was used to amplify a fragment of the HPV L1 gene. Every PCR plate included a negative (H₂O) and a positive (CaSki cell DNA) control to test for possible contamination. The Linear Array HPV Genotyping Test (Roche Diagnostics, Indianapolis, IN) was used to test for the presence of 37 HPV types, including 13 oncogenic types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 66) and 24 non-oncogenic types (6, 11, 26, 40, 42, 53, 54, 55, 61, 62, 64, 67–73, 81–84, IS39, and CP6108). A sample was considered HPV positive if HPV DNA was detected by PCR or it tested positive for at least one of the 37 HPV genotypes. Samples that amplified HPV DNA by PCR but did not test positive for a specific HPV genotype were considered unclassified infections. Beta-globin was detected in 93% (112/120) of GW samples. The eight men with β -globin negative GW samples were excluded from all analyses.

Statistical analysis

GW incidence rates were calculated by dividing the number of incident cases by the number of person-months of follow-up. Person-months were measured as the number of months from the date of enrollment until the date the incident GW was detected, or until the date of the last clinic visit for men who did not develop GW. Incidence rates were calculated for individual HPV types and groups of HPV types (non-oncogenic or oncogenic HPV types) detected on the surface of the GW. The 95% confidence intervals (CIs) for incidence rates were calculated based on the Poisson distribution [36]. All incidence rates were reported per 1,000 person-years.

The Kaplan-Meier method was used to estimate the 12 and 24 month cumulative incidence of GW and the corresponding 95% CIs overall, by age group, and among men with type specific incident HPV infections. Incident HPV infections were infections detected at a follow-up visit after a man tested negative for the same HPV type at enrollment. Men who did not develop GW were censored at the date of their last study visit. The log-rank test was used to test for differences in risk of GW by age group (18-30 years; 31-44 years; and 45-70 years) and by type of incident HPV infection (HPV 6/11 only; HPV 6/11 and other types; and HPV types other than 6/11). Among the 112 men who developed GW, the median time from type specific incident HPV infections to GW detection was calculated as time in months from the date an incident HPV infection was detected until the date the GW was detected.

Role of the funding source

Study sponsors had no role in the study design, data collection or analysis. The corresponding author had full access to all data in the study and had final responsibility for the decision to submit for publication.

RESULTS

Study participants were followed for a median of 17.9 months (range, 4.5-46.9; 25th – 75th percentiles, 7.0-29.6), with 112 incident cases of GW detected during follow-up. The mean age of participants was 32.6 years (standard deviation (SD), 11.4; range, 18-70), with 49% of men ages 18 to 30. Forty-five percent of men self-reported White race and 45.2% self-identified as Hispanic. At baseline, 64.8% of men tested positive for HPV DNA on the normal genital skin and 5.0% tested positive for HPV types 6 or 11 (data not shown).

Table 1 presents GW incidence rates and the distribution of HPV type groups (oncogenic versus non-oncogenic) detected on the surface of GW. The overall incidence rate for a newly acquired GW was 2.35 per 1,000 person-years and HPV DNA was detected in 80.4% of GW. Forty-two percent of GW had non-oncogenic HPV types only and HPV 6 and/or 11 was detected in 53.6% of the 112 incident GW and 66.7% of the 90 GW that tested positive for HPV DNA. Five percent of GW tested positive for oncogenic HPV types only and HPV 16/18 was detected in 12.5% of GW. Almost half of GW tested positive for multiple types of HPV (45.5%) and 27.7% of GW tested positive for a mix of oncogenic and non-oncogenic HPV types. Unclassified infections that tested positive for HPV DNA by PCR but did not hybridize a specific HPV type occurred in 5.4% of GW.

Table 2 presents HPV type specific incidence rates and the proportion of GW that tested positive for specific HPV types. Non-oncogenic types HPV 6 (43.8%) and HPV 11 (10.7%) were the most common types detected and had the highest incidence rates (1.03 per 1,000 person-years and 0.25 per 1,000 person years, respectively). All other HPV types were found in $\leq 10\%$ of GW and had incidence rates of < 1.0 per 1,000 person-years. Other common non-oncogenic HPV types detected were 62 (9.8%; 0.23 per 1,000 person-years) and 84 (8.9%; 0.21 per 1,000 person-years). The most

common oncogenic HPV types detected were 16 (9.8%; 0.21 per 1,000 person-years) and 52 (6.2%; 0.15 per 1,000 person-years). 58.9% of men who developed GW had an HPV infection at a study visit prior to GW development with one or more of the same HPV types detected on the surface of the GW. 65.3% of men with GW positive for HPV 6 and 58.3% of men with GW positive for HPV 11 had a preceding HPV infection with types 6 and 11, respectively (data not shown).

The cumulative risk of developing GW was 1.7% (95% CI: 1.2-2.3) at 12 months and 5.2% (95% CI: 4.1-6.4) at 24 months and the median time until detection of any GW regardless of HPV status was 17.1 months (95% CI: 12.4-19.3 months) (Table 3). Among men with an incident HPV infection with any type, cumulative incidence of GW was 2.4% (95% CI: 1.6-3.3) at 12 months and 6.8% (95% CI: 5.0-8.6) at 24 months. Though not statistically significant, cumulative incidence at 12 months was higher among men with incident HPV infections with non-oncogenic types only (4.1%; 95% CI: 2.2-6.0) compared to men with incident infections with oncogenic types only (1.8%; 95% CI: 0.0-3.5), or a mix of oncogenic and non-oncogenic types (1.5%; 95% CI: 0.5-2.4) ($p=0.39$). Men with an incident HPV 6/11 infection had the highest probability of developing GW (Figure 1). Twelve months after an incident HPV infection, 8.9% (95% CI: 0.0-18.1) of men with HPV 6/11 only, 5.2% (95% CI: 1.8-8.5) of men with HPV 6/11 and other types, and 2.5% (95% CI: 1.5-3.4) of men with HPV types other than 6/11 developed GW. The probability of developing GW over 24-months was significantly higher among men with an HPV infection that included HPV types 6/11 (14.6%; 95% CI: 7.5-21.1) than men with an HPV infection not positive for types 6/11 (5.5%; 95% CI: 3.8-7.3) ($p<0.0001$) (Figure 1, Table 3). Time to GW detection was also shorter among men with an HPV infection with type 6/11 only (6.2 months, 95% CI: 5.6-24.2), and among men with an HPV infection with 6/11 and other HPV types (13.3 months; 95% CI: 6.3-19.6) than among

men with an HPV infection with HPV types other than 6/11 (18.2 months; 95% CI: 12.4-23.6).

Incidence of GW significantly varied across age groups (p-value <0.0001) (Figure 2). Although the 24 month cumulative incidence of GW was highest in younger men (7.4%; 95% CI: 5.4-9.4), mid adult (3.1%; 95% CI: 1.6-4.6) and older men (3.3%; 95% CI: 0.9-5.6) remained at risk for GW and the shortest time to GW detection was observed among men ages 45-70 (Table 4). There were no significant differences in GW incidence across countries within each age group (data not shown).

DISCUSSION

In this multinational cohort of men ages 18-70 we estimated GW incidence, time from HPV infection to GW detection, and described the distribution of HPV types detected on the surface of incident GW. Men with incident HPV 6/11 infections had the highest incidence of GW and shortest time from HPV infection to GW detection. HPV 6 (43.8%) and 11 (10.7%) were the most common types detected on GW, but there was also a high prevalence of oncogenic types including HPV 16 (9.8%).

The incidence rate of GW among all men in our study was 2.35 per 1,000 person-years, with the highest incidence of 3.43 per 1,000 person-years observed among men ages 18-30. Our findings are similar to incidence estimates from studies using private health insurance claims that reported GW incidence rates of 1.70 [16], 1.62 [17], and 1.10 [15] per 1,000 person-years. Those studies also observed the highest GW incidence among younger men. Two studies observed peak GW incidence among men ages 25-29 (5.01 per 1,000 person-years [15] and 2.7 per 1,000 person-years [16]), and one study observed the highest incidence among men ages 20-29 (3.1 per 1,000 person-years [17]). Higher GW incidence rates have been observed among individuals in the placebo arms of HPV vaccine clinical trials: 8.7 per 1,000 person-years among

females ages 15-26 [24] and 15.8 per 1,000 person-years among males ages 16-26 [32]. An enrollment criterion for the clinical trials was having four or fewer lifetime sexual partners. The median number of lifetime sexual partners in the current study was six, thus the lower GW incidence observed in the current study versus the clinical trials is unexpected. One possible explanation is that young individuals just beginning to be sexually active, and therefore exposed to HPV for the first time, have not developed the immune response to clear an HPV infection and therefore are at higher risk of developing GW. Time to clearance of an HPV infection is significantly longer in younger men [3], who may consequently have a greater likelihood of developing a lesion.

Approximately 15% of men in the current study developed GW within 24 months of an incident HPV 6/11 infection. This is lower than a cohort of university students in which 58% of males [18] and approximately 60% of females [23] developed GW within 24 months of an incident HPV 6/11 infection. The age distribution of participants in each study may partially account for the difference. The student cohort only included individuals 18-21 while our study included men ages 18-70. However, the 24 month cumulative incidence of GW after an incident HPV 6/11 infection was only 22.5% among men ages 18-21 in our study. Differences in time intervals between clinic visits may also contribute to our lower observed GW incidence. Men in our study had a slightly longer time of six months between visits compared to the cohort of students who were examined every four months. Given that the median time to clearance of GW was 5.9 months in the female students [23], it is possible there were men in our study who developed and cleared an incident GW between the 6-month clinic visits.

Among men in our study with an incident HPV 6/11 infection, the median time to GW detection was 12.2 months, similar to the median time of 11.0 months reported among male university students with an incident HPV 6/11 infection [18]. Women appear to have a shorter time from HPV 6/11 infections to GW detection; the placebo

arm of a vaccine trial in women reported median times of 5.0 months [24] and the study of female university students reported a median time of 2.9 months [23]. It is not known why GW develop more slowly in men, but this observation is consistent with findings of peak GW incidence occurring at a slightly older age in men than in women [15, 16].

The prevalence of HPV 6/11 in GW in our study was 54%. Previous studies reported the HPV 6/11 prevalence in GW to be 86% among young women in the placebo arm of an HPV vaccine trial [24], 89% in men from Hong Kong [26], 90% among French men ages 18-72 [27], and greater than 95% in two small US studies that included fewer than 50 men [28, 33]. The lower than expected prevalence of HPV 6/11 observed in GW in the current study may be the result of misclassification. Preliminary biopsy data collected from a small sample of lesions identified in the HIM Study found 39% of lesions diagnosed as GW by visual inspection were not true GW by pathology. The high rate of false positives suggests that non-condyloma skin conditions were classified as condyloma based on visual inspection and therefore were not positive for HPV 6/11. We also observed a high proportion of oncogenic HPV types in GW (33.1%), with HPV 16 (9.8%) being the most common type detected after HPV 6 (43.8%) and HPV 11 (10.7%). This finding is consistent with other studies that also reported a high prevalence of oncogenic HPV types in GW [24, 26, 27, 33].

The major limitation of this study was reliance on visual inspection to identify GW. Without pathological confirmation that lesions were GW, it is possible that non-HPV related skin conditions were incorrectly classified as GW. The lower than expected prevalence of HPV 6/11 and the slightly higher incidence rates of GW compared to previous studies [15-17] suggests some misclassification is present. The generalizability of our findings may also be limited, since men who agree to participate in a four-year study are likely not representative of the underlying population at each study site. However, our findings are likely more generalizable to a broader population than findings

from clinical trials, which have a more select group of individuals due to more stringent selection criteria. Also, HPV detection in the current study was based on samples obtained by sampling the surface of the GW, and therefore, the types detected may not represent the types present in the lesions themselves. However, a small sample of histologically confirmed condyloma from the HIM Study found swabbing the surface of the lesion was a highly sensitive and specific method for detecting the HPV types present within the condyloma tissue [37].

The major strength of the current study was the longitudinal study design and long duration of follow-up. Repeated measures of HPV status over follow-up enabled the examination of how time to GW development differed after incident HPV infections with specific types. We also included men from a broader age range than most previous studies, which allowed us to examine how incidence of GW differed with age.

This study is one of the first to examine progression from HPV infection to GW including men from across the lifespan. Though younger men had the highest incidence of GW mid-adult and older men still remained at risk of acquiring GW. HPV 6/11 appears to play an important role in GW development with the highest incidence and shortest time to GW development observed among men with incident HPV 6/11 infections. Future studies should confirm these incidence estimates among histologically confirmed GW.

Table 2.1. Genital wart incidence by grouped HPV types detected on the surface of the lesion.

HPV type detected on surface of GW	n (%) ^a	Incidence per 1,000 person-years (95% Confidence Interval)
Incidence of GW regardless of HPV type detected on the lesion	112 (100.0)	2.35 (1.94-2.83)
Positive for HPV ^b	90 (80.4)	1.89 (1.52-2.33)
Non-oncogenic HPV types only	47 (42.0)	0.99 (0.73-1.31)
HPV 6/11	60 (53.6)	1.26 (0.96-1.62)
Oncogenic HPV types only	6 (5.4)	0.13 (0.05-0.27)
HPV 16/18	14 (12.5)	0.29 (0.16-0.49)
Both non-oncogenic and oncogenic HPV types	31 (27.7)	0.65 (0.44-0.93)
Positive for multiple HPV types	51 (45.5)	1.05 (0.78-1.39)
Unclassified infections ^c	6 (5.4)	0.13 (0.05-0.27)

^aDenominator is the 112 men who developed incident GW.

^bIncludes unclassified HPV infections.

^cInfections that tested positive for HPV DNA by PCR but did not test positive for any of the 37 HPV types.

Table 2.2. Genital wart incidence by individual HPV types detected on the surface of the lesion.

	No. men who developed GW with HPV type n (%) ^{a,b}	Incidence of GW per 1,000 person-years (95% Confidence Interval)
Non-oncogenic HPV		
6	49 (43.8)	1.03 (0.76-1.36)
11	12 (10.7)	0.25 (0.13-0.44)
26	0 (0.0)	-
40	7 (6.2)	0.15 (0.06-0.30)
42	6 (5.4)	0.13 (0.05-0.27)
53	8 (7.1)	0.17 (0.07-0.33)
54	5 (4.5)	0.11 (0.03-0.25)
55	7 (6.2)	0.15 (0.06-0.30)
61	2 (1.8)	0.04 (0.01-0.15)
62	11 (9.8)	0.23 (0.12-0.41)
64	0 (0.0)	-
67	1 (0.9)	0.02 (0.00-0.12)
68	3 (2.7)	0.06 (0.01-0.18)
69	0 (0.0)	-
70	0 (0.0)	-
71	2 (1.8)	0.04 (0.01-0.15)
72	2 (1.8)	0.04 (0.01-0.15)
73	1 (0.9)	0.02 (0.00-0.12)
81	1 (0.9)	0.02 (0.00-0.12)
82	1 (0.9)	0.02 (0.00-0.12)
83	2 (1.8)	0.04 (0.01-0.15)
84	10 (8.9)	0.21 (0.10-0.39)
IS39	0 (0.0)	-
CP6108	7 (6.2)	0.15 (0.06-0.3)
Oncogenic HPV		
16	11 (9.8)	0.21 (0.10-0.39)
18	4 (3.6)	0.08 (0.02-0.22)
31	1 (0.9)	0.02 (0.00-0.12)
33	0 (0.0)	-
35	0 (0.0)	-
39	5 (4.5)	0.11 (0.03-0.25)
45	1 (0.9)	0.02 (0.00-0.12)
51	6 (5.4)	0.13 (0.05-0.27)
52	7 (6.2)	0.15 (0.06-0.30)
56	1 (0.9)	0.02 (0.00-0.12)
58	4 (3.6)	0.08 (0.02-0.22)
59	5 (4.5)	0.11 (0.03-0.25)
66	6 (5.4)	0.13 (0.05-0.27)

^aDenominator is the 112 men who developed incident GW.

^bPercentages do not sum to 100 due to men having multiple HPV types and being included in multiple HPV categories..

Table 2.3. Cumulative incidence of genital warts at 12 and 24 months and median time from incident HPV infection to genital wart detection.

Incident HPV infection genotype ^a	12 month cumulative incidence, % (95% CI)	24 month cumulative incidence, % (95% CI)	Median time in months from HPV infection ^a to GW detection (95% CI)
Incidence of GW regardless of HPV status	1.7 (1.2-2.3) ^b	5.2 (4.1-6.4) ^b	17.1 (12.4-19.3) ^c
Negative for HPV	1.1 (0.1-2.2)	1.8 (0.1-3.5)	N/A
Positive for HPV	2.4 (1.6-3.3)	6.8 (5.0-8.6)	12.6 (12.1-18.4)
Non-oncogenic HPV types only	4.1 (2.2-6.0)	6.7 (3.9-9.4)	7.6 (6.2-12.2)
Oncogenic HPV types only	1.8 (0.0-3.5)	5.5 (0.9-9.9)	19.4 (6.2-23.5)
Both non-oncogenic and oncogenic HPV types	1.5 (0.5-2.4)	6.8 (4.3-9.3)	18.8 (12.7-23.9)
IHPV 6/11 ^d	5.8 (2.6-9.0)	14.6 (7.5-21.1)	12.2 (6.2-18.9)
HPV 6/11 only	8.9 (0.0-18.1)	13.7 (0.0-25.8)	6.2 (5.6-24.2)
HPV 6/11 and other HPV types	5.2 (1.8-8.5)	14.5 (6.7-21.7)	13.3 (6.3-19.6)
HPV types other than 6/11	2.5 (1.5-3.4)	5.5 (3.8-7.3)	18.2 (12.4-23.6)

Note: CI-confidence interval.

^aIncident HPV infection that occurred before development of GW.

^bCumulative incidence of GW among all 2,487 men regardless of HPV infection status.

^cMedian time from enrollment visit to GW detection.

^dIncludes incident HPV infections with 6/11 only and infections with 6/11 and other HPV types.

Table 2.4. Cumulative incidence of genital warts at 12 and 24 months and median time to genital wart detection by age groups.

Age group (years)	Incidence per 1,000 person-years (95% CI)	12 month cumulative incidence-% (95% CI)	24 month cumulative incidence-% (95% CI)	Median time in months from incident HPV infection to GW detection (95% CI)
18-30	3.43 (2.72-4.27)	2.3 (1.4-3.2)	7.4 (5.4-9.4)	17.2 (12.3-19.5)
31-44	1.37 (0.89-2.02)	1.0 (0.3-1.7)	3.1 (1.6-4.6)	18.9 (11.9-24.2)
45-70	1.27 (0.55-2.51)	1.7 (0.2-3.2)	3.3 (0.9-5.6)	7.6 (5.8-13.6)

Note: CI-confidence interval.

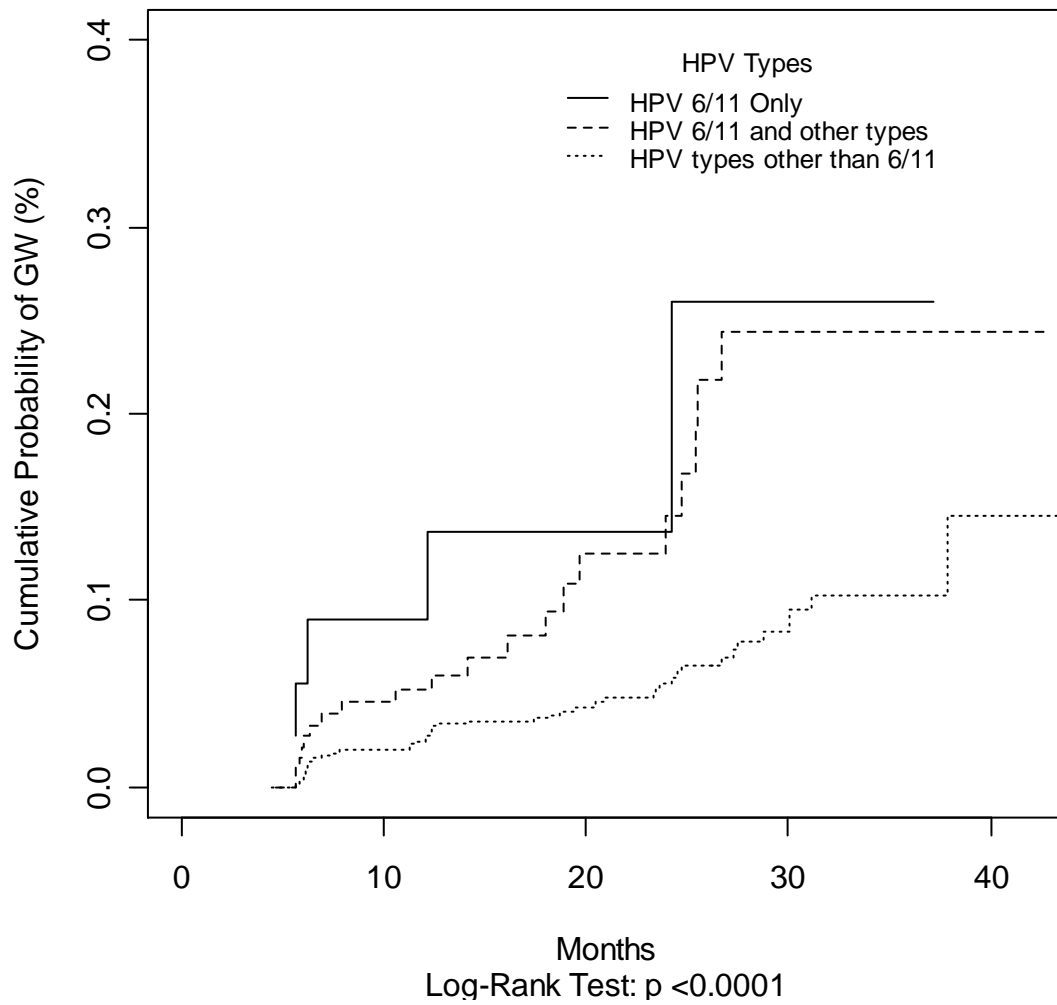


Figure 2.1. Cumulative probability of genital warts among men with incident HPV infections with HPV 6/11 only, HPV6/11 and other HPV types, and only HPV types other than 6/11.

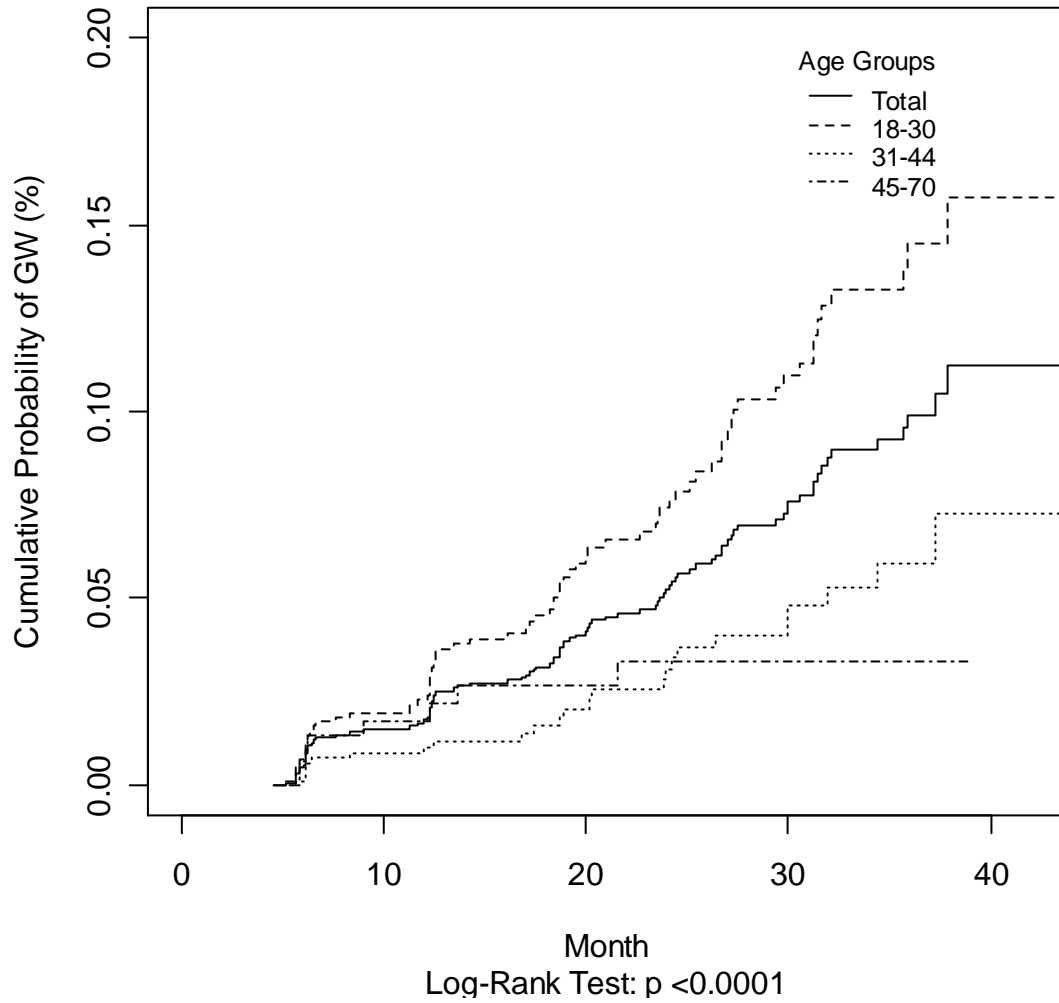


Figure 2.2. Cumulative probability of genital warts by age groups.

CHAPTER 3: SECOND MANUSCRIPT: RISK FACTORS FOR CONDYLOMA IN A MULTINATIONAL COHORT OF MEN: THE HIM STUDY

ABSTRACT

Background: Little is known about the sociodemographic and sexual behavior factors associated with incident genital warts (GW) in men.

Methods: A cohort of 2,487 men from the US, Brazil and Mexico were followed every 6-months for a median of 17.9 months. At each study visit men completed a questionnaire to obtain information on sexual behavior and a trained clinician identified GW by visual inspection and sampled for the presence of human papillomavirus (HPV). Cox proportional hazards models were used to examine factors independently associated with incident GW.

Results: Factors associated with GW were incident HPV 6/11 infection (hazard ratio (HR)=12.42; 95% confidence interval (CI): 3.78-40.77), younger age (HR=0.43; 95% CI: 0.26-0.77; 45-70 vs. 18-30 years), high lifetime number of female partners (HR=5.69; 95% CI: 1.80-17.97); ≥ 21 vs. 0), and sexual behaviors in the previous three months including infrequent condom use (HR=2.44; 95% CI: 1.16-5.14; <half the time vs. always), number of male sexual partners (HR=4.53; 95% CI: 1.68-12.20; ≥ 3 vs. none), frequent vaginal intercourse (HR=4.14; 95% CI: 1.32-13.01); ≥ 21 times vs. none), having a partner with GW (HR=2.38; 95% CI: 1.01-5.61), and being diagnosed with a sexually transmitted infection (HR=1.99; 95% CI: 1.17-3.39).

Conclusion: HPV 6/11 and recent sexual behavior were most strongly associated with incident GW in men.

INTRODUCTION

Genital warts (GW) are one of the most prevalent sexually transmitted infections (STI) in the United States (US), and the incidence of GW has been increasing in the last decade [17]. Approximately 90% of GW are associated with human papillomavirus (HPV), particularly non-oncogenic HPV types 6 and 11 [27]. Though GW are not associated with mortality, they are a source of emotional distress and reduced quality of life [9, 10, 38]. GW have a high transmission rate between sexual partners [11], and treatment of GW is often ineffective with about one-fourth of cases recurring within 3 months of treatment [12]. Identifying factors associated with GW can contribute to prevention efforts that focus on behavioral modification.

Only a few studies have examined the factors associated with the development of GW in men [19-22], and most of these studies were among highly selective populations including STI clinic attendees [20, 21] and men who have sex with men [22]. Similarly, many studies examining risk factors for GW in women have also been in select populations such as university students [23], STI clinic attendees [20], and young women in the placebo arm of an HPV vaccine trial [24]. The purpose of this study was to identify sociodemographic and sexual behavioral factors associated with the incidence of GW in a cohort of men ages 18-70 residing in the US, Brazil, and Mexico.

METHODS

Study population

The HPV in Men (HIM) Study is a multinational prospective study of men ages 18-70 that examines the natural history of HPV infection in men. Participants were enrolled between July 2005 and September 2009 and met the following inclusion criteria: (a) were ages 18-70 years old; (b) resided in Southwest Florida, Sao Paulo, Brazil or the state of Morelos Mexico;

(c) reported no previous diagnosis of penile or anal cancer; c no prior diagnosis of genital or anal warts; (e) had not participated in an HPV vaccine clinical trial; (f) reported no prior diagnosis of HIV or AIDS; (g) were not currently being treated for an STI; (h) had not been imprisoned, homeless or in drug treatment in the previous six months; and (i) were willing to complete 10 scheduled visits every six months over four years.

The current analysis included the first 2,487 men enrolled in the HIM study through January 1, 2009 who did not have GW detected at enrollment and completed at least one 6-month follow-up visit. All participants provided written informed consent and study protocols were approved by Institutional Review Boards at each study site.

Study design

Men completed a pre-enrollment run-in visit followed by an enrollment visit approximately two weeks later. After enrollment, men returned approximately every six months for eight additional follow-up visits. At each study visit participants completed an extensive risk factor questionnaire in their native language (English, Spanish, or Portuguese), administered using Computer-Assisted Self-Interviewing. The survey obtained information about sociodemographic factors and lifetime and recent sexual behavior.

At each clinic visit a trained clinician examined men for genital lesions. GW were lesions with a wart-like architecture that did not appear to be related to Herpes Simplex virus or a benign condition such as pearly penile papules, skin tags, cysts, or Fordyce spots. Saline-prewettted Dacron swabs were used to obtain samples of healthy penile epithelium from the coronal sulcus/glans penis, penile shaft, and scrotum. The three samples were combined for HPV DNA testing and genotyping. A full description of study procedures has been published previously [3, 4, 35].

HPV DNA testing

Polymerase chain reaction (PCR) was used to test for HPV DNA. Following the instructions of the manufacturer (Qiagen, Valencia, CA), the QIAamp Mini kit was used to extract DNA from the skin swabs. A fragment of the HPV L1 gene was amplified using the PCR consensus primer system PGMY 09/11. The Linear Array HPV Genotyping Test (Roche Diagnostics, Indianapolis, IN) was used to test for 37 HPV types, including 13 oncogenic types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 66) and 24 non-oncogenic types (6, 11, 26, 40, 42, 53, 54, 55, 61, 62, 64, 67–73, 81–84, IS39, and CP6108). HPV genotyping was conducted on all samples, regardless of HPV PCR result. A sample was classified as HPV positive if HPV DNA was detected by PCR or the sample tested positive for at least one of the 37 HPV genotypes tested for.

Statistical analysis

The Pearson's chi-squared test for categorical variables and the t-test for continuous variables were used to compare the baseline distribution of sociodemographic characteristics and sexual behavior factors between men with incident GW and men who did not develop GW. Cox proportional hazard models were used to calculate hazard ratios (HRs) and 95% confidence intervals (CIs) for the association between incident and prevalent HPV infection and risk of GW. An individual had an incident HPV infection for a specific HPV type if he tested negative for that type at enrollment and later tested positive for the same type at a follow-up visit. Prevalent HPV infections were infections present at enrollment. The reference group for all models assessing the association between HPV infection and GW was men who tested negative for HPV at all study visits. Person-time was calculated as the months from the enrollment date until the date of the visit at which a GW was detected or until the last follow-up visit for men who did not develop GW.

Cox proportional hazard models were also used to examine crude and multivariable associations between sociodemographic and sexual behavior factors and the risk of developing GW. The backward selection method, with a significance threshold of 0.05, was used to determine the factors included in the final multivariable model. Variables initially included were: race, ethnicity, marital status, education, cigarette smoking status, circumcision status, age at first intercourse with a female, lifetime and recent number of female and male sexual partners, sexual orientation, condom use, frequency of vaginal intercourse, having a steady female partner, ever being diagnosed with an STI, ever having a partner with an STI, ever having a partner with GW, and incident HPV 6/11 infection. Country of residence (US; Brazil; Mexico) and age (18-30 years; 31-44 years; 45-70 years) were study design factors and therefore included in all multivariable models. Covariates that could change over the follow-up period, (e.g., recent number of female partners), were treated as time-dependent variables. The final multivariable model was run including among all men in the cohort (n=2487) and then restricted to men who had an incident HPV infection of any type during follow-up (n=1498). All p-values were 2-sided and considered significant if below 0.05. Statistical analyses were conducted using SAS version 9.1.

RESULTS

Men were followed for a median of 17.9 months (range, 4.5-46.9; 25th – 75th percentiles, 7.0-29.6), and 112 men developed GW. Table 1 compares the baseline distribution of sociodemographic and sexual behavior factors between men who developed GW and men who did not. Compared to men who did not develop GW, men with incident GW were significantly younger, more likely to be White, current smokers, circumcised, have a high number of lifetime female sexual partners, have more female sexual partners in the previous three months, and not always use condoms.

Table 2 presents the associations between types of HPV infections and risk of subsequent GW. The strongest associations were observed for infections with non-oncogenic HPV types 6/11. Compared to men who never tested positive for HPV, there was a significant increased risk for GW among men with an incident HPV infection with types 6/11 only (HR=12.42; 95% CI: 3.78-40.77) and among men with an incident infection with HPV 6/11 and other types (HR=7.74; 95% CI: 3.10-19.31). Risk of GW was also significantly higher among men with an incident infection with non-oncogenic HPV types only (HR=3.63; 95% CI: 1.49-8.83) or a mix of non-oncogenic and oncogenic types (HR=3.94; 95% CI: 1.68-9.27). There was no significant increased risk for GW among men with incident infections with oncogenic HPV types only (HR=2.42; 95% CI: 0.56-6.86) or men with incident infections with HPV types other than 6/11 (HR=2.16; 95% CI: 0.93-5.02). Similar associations were observed for prevalent HPV infections at enrollment, with the highest risk for GW among men with an HPV infection with types 6/11 only (HR=16.78; 95% CI: 5.97-47.19).

Table 3 presents the risk of GW for factors that remained in the final multivariable model, while adjusting for HPV 6/11 infection, for the entire cohort (n=2,487) and restricted to men with an incident HPV infections (n=1,498). For the entire cohort, the factors independently associated with risk of GW, while adjusting for infection with HPV 6/11, were country, age, lifetime number of female sexual partners, recent condom use, recent number of male anal sex partners, frequency of vaginal intercourse in the past three months, ever having a partner with GW, and ever being diagnosed with an STI. Compared to men residing in the US, the risk of GW was lower for men living in Brazil (HR=0.33; 95% CI: 0.20-0.54) and Mexico (HR=0.45; 95% CI: 0.26-0.77). Risk of GW decreased with age and was comparable among men ages 31-44 (HR=0.44; 95% CI: 0.27-0.71) and 45-70 (HR=0.43; 95% CI: 0.20-0.92) compared to men ages 18-30. Compared to men who reported no female sexual partners in their lifetime, risk of GW

increased with an increasing number of female partners (p for trend: <0.0001). The significant increase in risk however was only among men with six or more lifetime female partners. Risk of GW was also significantly higher among men who refused to report lifetime number of female partners. However, including these men in the model did not bias results, as there were no major differences in risk estimates when the multivariable model was run after excluding these 135 men (data not shown). Sexual behaviors in the previous three months that were associated with an increased risk of GW were a high number of male anal sex partners (HR=4.53; 95% CI: 1.68-12.20 for men who reported three or more recent male partners compared to men with no recent male partners), more frequent vaginal intercourse (HR=4.14; 95% CI: 1.31-13.01 for ≥ 21 times compared to men who reported no vaginal intercourse in the recent past), and infrequent condom use (HR=2.44; 95% CI: 1.16-5.14 for using condoms less than half the time vs. always). Ever being diagnosed with an STI (HR=1.99; 95% CI: 1.17-3.39) and ever having a partner with GW (HR=2.38; 95% CI: 1.01-5.61) also increased the risk of GW.

The multivariable model was also run restricted to men with an incident HPV infection to examine which factors in addition to HPV were associated with incident GW (Table 3). Factors that remained significantly associated with GW were country, age, lifetime number of female partners, recent condom use, and being diagnosed with an STI. Recent number of male anal sex partners, frequency of vaginal intercourse in the previous three months, and having a partner with GW were not significantly associated with risk of GW among men with an incident HPV infection.

DISCUSSION

In this cohort of men ages 18-70 from the US, Brazil, and Mexico, HPV 6/11 infections were most strongly associated with incidence of GW. Recent sexual behaviors associated with incidence of GW were condom use, recent number of male

anal sex partners, frequency of vaginal intercourse, having a partner with GW, and testing positive for an STI. This is the first study to use a prospective design to examine sexual behaviors associated with GW, thus minimizing recall bias that may have occurred in previous case-control studies. The current study also includes men largely from the general community, making the results more generalizable than previous studies which included men from more select populations.

In our cohort, men with incident HPV infections with only types 6/11 had the highest risk of developing GW. Previous analyses of this cohort of men also found the highest incidence of GW and shortest time from HPV infection to GW detection among men with incident HPV 6/11 infections (data not shown). The strong association between HPV 6/11 and GW has also been observed among females enrolled in the placebo arm of an HPV vaccine trial [24]; women who tested positive for HPV 6/11 at baseline were 29 times more likely to develop GW in the first year of follow-up compared to women negative for HPV 6/11. The same study saw a significant increased risk for GW among women who had HPV infections with oncogenic types only. We also saw an increased risk of GW among men with HPV infections with oncogenic types only at enrollment. It is likely that the men with oncogenic infections at enrollment acquired a subsequent non-oncogenic HPV infection prior to GW development.

Consistent with the nature of a sexually transmitted disease, the risk of GW was highest among men with a high lifetime number of female sexual partners or more frequent vaginal intercourse. The association between a high lifetime number of female partners and risk of GW was also observed in STI clinic attendees [20] and male members of a health maintenance organization [19]. A higher number of sexual partners and more frequent sexual intercourse increase a man's chance of being exposed to HPV. Having a high number of female partners [3, 35, 39, 40] and frequent sexual intercourse [39] are both significantly associated with HPV infection in men. We also

observed a significant increased risk for GW among men who refused to report their lifetime number of female partners. These men likely had a high number of partners but did not provide an answer because they were not sure of the exact number or they were embarrassed to report a large number.

Always using condoms was protective against GW in our cohort. Frequent condom use was also protective against GW in a study of STI clinic attendees [20], but no association was observed in a study of male health maintenance organization members [19]. Similarly, there have been inconsistent findings on the protective effect of condom use against HPV infection in men [41]. Condoms provide a protective barrier against the transmission of HPV by skin to skin contact; however, men can be infected with HPV on areas not protected by a condom. We did not observe an increased risk for GW among the men who reported never using condoms in the recent past. Frequent condom use may be a marker for engaging in high risk sexual behavior such as having multiple partners, while not using condoms may be a marker for low risk behavior such as being in a monogamous relationship. The increased risk of GW among men reporting no vaginal sex in the last three months was likely due to the fact that this category included men who had one or more male anal sex partners in the recent past.

Men who had three or more male anal sex partners in the previous three months had a significantly increased risk of GW compared to men with no recent male partners. This observation is consistent with results from a male vaccine trial in which men who had sex with men had an incidence rate of GW more than twice as high as men who only had sex with women [32]. However, lifetime number of male sexual partners was not associated with GW in our study. We also did not observe an increased risk for GW among men with a high number of recent female partners, consistent with a study of STI attendees that found no association between GW and the number of sexual partners in the previous 12 months [21]. Having had a partner with GW significantly increased the

risk for GW. This observation is consistent with the high transmission rate of GW between partners [11] and a finding that men with a female partner with GW are more likely to be HPV positive [42].

Though age is not associated with incidence of HPV infection in men [3], we found that the risk of GW significantly decreased with age independent of sexual behavioral factors including lifetime number of female partners. This age pattern has consistently been observed in other studies examining risk factors for GW in men [19-22] as well as GW incidence estimates based on data from US insurance claims [15-17]. Changes in immune response with increasing age may be related to the lower incidence of GW in older men. Though the prevalence of HPV in men remains steady across the lifespan, older men clear HPV infections faster than younger men [3] and increasing age is associated with higher levels of antibodies against HPV types 6 and 11 [43]. More rapid clearance and a stronger immune response may reduce the likelihood that an HPV infection progresses to a lesion.

There was a significantly reduced risk of GW among men in Brazil and Mexico compared to men in the US. The difference in risk across countries may be partially due to residual confounding by age; country remained a significant factor when the final multivariable model was run among men ages 18-30, but the association with country did not remain significant in models restricted to men ages 31-44 or 45-70 (data not shown).

There are limitations to the current study that should be considered when interpreting the results. GW were identified by visual inspection, therefore misclassification may exist if lesions we classified as GW were actually a non-HPV related benign skin condition. However, misclassification of GW would likely be non-differential with respect to sexual behavior and, therefore, result in underestimates of the associations between GW and various risk factors. The generalizability of our findings is

likely limited due to the self-selection of participants. Men who agree to participate in a four year prospective study may not be representative of the underlying population from each country. However, our results are likely more generalizable than studies that only included men who have sex with men or men who were seeking treatment for an STI.

Strengths of our study included use of an extensive questionnaire to collect data for a variety of potential risk factors and a prospective study design that allowed us to obtain data on sexual behavior before men developed GW. Previous studies of risk factors for GW in men were case-control studies that collected risk factor data after men were diagnosed with GW, potentially leading to biased results if being diagnosed with GW caused men to alter their sexual behavior (e.g., use condoms more frequently) or affected how accurately they recalled their sexual habits. By collecting data on lifetime and recent sexual behavior before men were diagnosed with GW, recall bias was minimized.

In summary, the factors independently associated with an increased risk of GW in this cohort of men ages 18-70 included incident HPV 6/11 infection, a high number of lifetime female or recent male sexual partners, frequent vaginal intercourse, infrequent condom use, having with a partner with GW and ever being diagnosed with an STI. The strong association between recent sexual history and incident condyloma after accounting for HPV infection suggests that prevention efforts targeting behavioral modification may be effective at reducing condyloma incidence among men who are not vaccinated.

Table 3.1. Distribution of sociodemographic and sexual behavior characteristics of cohort members at enrollment.

	Genital Warts (N=112) n (%)	No Genital Warts (N=2375) n (%)	p-value ^a
Country			
United States	61 (54.5)	657 (27.7)	<0.0001
Brazil	31 (27.7)	935 (39.4)	
Mexico	20 (17.9)	783 (33.0)	
Age			
18-30	79 (70.5)	1142 (48.1)	<0.0001
31-44	25 (22.3)	903 (38.0)	
45-70	8 (7.1)	330 (13.9)	
Mean (SD)	28.5	32.8	<0.0001
Race			
White	70 (62.5)	1046 (44.0)	<0.01
Black	16 (14.3)	385 (16.2)	
Asian/Pacific Islander	0 (0.0)	59 (2.5)	
American Indian	1 (0.9)	54 (2.3)	
Mixed/Mestizo	24 (21.4)	802 (33.8)	
Unknown/Refused	1 (0.9)	29 (1.2)	
Current smoker			
Yes	27 (24.1)	500 (21.1)	0.04
No	84 (75.0)	1873 (78.9)	
Refused	1 (0.9)	2 (0.1)	
Circumcision (clinician assessed)			
Yes	65 (58.0)	774 (32.6)	<0.0001
No	47 (42.0)	1601 (67.4)	
Sexual orientation at enrollment			
Men who have sex with women only	98 (87.5)	1971 (83.0)	0.18
Men who have sex with women and men	6 (5.4)	129 (5.4)	
Men who have sex with men only	5 (4.5)	113 (4.8)	
Never had sex	2 (1.8)	157 (6.6)	
Refused	1 (0.9)	5 (0.2)	
Lifetime no. of female sexual partners			
0	5 (4.5)	254 (10.7)	<0.01
1	6 (5.4)	205 (8.6)	
2 to 5	20 (17.9)	654 (27.5)	
6 to 10	28 (25.0)	463 (19.5)	
11 to 20	21 (18.8)	353 (14.9)	
≥21	24 (21.4)	319 (13.4)	
Refused	8 (7.1)	127 (5.4)	
Total no. of female partners in past 3 months			
None	17 (15.2)	813 (34.2)	<0.0001
1	49 (43.8)	934 (39.3)	
2	23 (20.5)	288 (12.1)	
≥3	21 (18.8)	271 (11.4)	
Refused	2 (1.8)	69 (2.9)	
No. of new female partners in past 3 months			
None	63 (56.3)	1506 (63.4)	0.08
1	29 (25.9)	523 (22.0)	
2	9 (8.0)	124 (5.2)	
≥3	9 (8.0)	106 (4.5)	
Refused	2 (1.8)	116 (4.9)	

Table 3.1. Distribution of sociodemographic and sexual behavior characteristics of cohort members at enrollment (Contd.).

	Genital Warts (N=112) n (%)	No Genital Warts (N=2375) n (%)	p-value ^a
Condom use during vaginal intercourse in past 3 months			
Always	27 (24.1)	436 (18.4)	0.03
At least half the time	30 (24.8)	424 (17.9)	
<Half the time	16 (14.3)	279 (11.8)	
Never	26 (23.2)	732 (30.8)	
No vaginal sex in the past 3 months	13 (11.6)	475 (20.0)	
Refused	0 (0.0)	29 (1.2)	
No. of times of vaginal intercourse in past 3 months			
None	19 (17.0)	696 (29.3)	0.07
1 to 5	22 (19.6)	342 (14.4)	
6 to 20	30 (26.8)	566 (23.8)	
≥21	35 (31.3)	647 (27.2)	
Refused	6 (5.4)	124 (5.2)	
Lifetime no. of male anal sex partners			
None	97 (86.6)	2013 (84.8)	0.80
1	3 (2.7)	99 (4.2)	
2	3 (2.7)	61 (2.6)	
≥3	7 (6.3)	180 (7.6)	
Refused	2 (1.8)	22 (0.9)	
No. of male anal sex partners in past 3 months			
None	105 (93.8)	2218 (93.4)	0.70
1	3 (2.7)	54 (2.3)	
2	0 (0.0)	25 (1.1)	
≥3	4 (3.6)	64 (2.7)	
Refused	0 (0.0)	14 (0.6)	
Ever had a partner with an STI			
Yes	27 (24.1)	372 (15.7)	0.12
No	48 (42.9)	1181 (49.7)	
Don't know	37 (33.0)	821 (34.6)	
Refused	0 (0.0)	1 (0.1)	
Ever had a partner with genital warts			
Yes	9 (8.0)	120 (5.1)	0.34
No	70 (62.5)	1582 (66.6)	
Don't know	33 (29.5)	673 (28.3)	
Refused	0 (0.0)	0 (0.0)	
Ever been diagnosed with an STI by a doctor			
Yes	22 (19.6)	387 (16.3)	0.08
No	86 (76.8)	1923 (81.0)	
Don't know	3 (2.7)	63 (2.7)	
Refused	1 (0.9)	2 (0.1)	

^aDifferences between groups was tested with the chi-squared test for categorical variables and t-test for continuous variables.

Table 3.2. Independent association between HPV infection and risk of genital warts.

	Genital Warts (n=112) n (%)	No Genital Warts (n=2375) n (%)	Hazard Ratio (95% Confidence Interval)
No HPV infection ^a	6 (5.4)	410 (17.3)	1.00 (ref)
INCIDENT HPV INFECTIONS			
Any HPV type	80 (71.4)	1418 (59.7)	3.80 (1.65-8.73)
Non-Oncogenic HPV types only	27 (24.0)	527 (22.2)	3.63 (1.49-8.83)
Oncogenic HPV types only	9 (8.0)	290 (12.2)	2.42 (0.56-6.86)
Both non-oncogenic and oncogenic types	44 (39.3)	601 (25.3)	3.94 (1.68-9.27)
HPV 6/11 ^b	25 (22.3)	199 (8.4)	7.95 (3.25-19.43)
HPV 6/11 only	5 (4.5)	31 (1.3)	12.42 (3.78-40.77)
HPV 6/11 and other HPV types	20 (17.9)	168 (7.1)	7.74 (3.10-19.31)
HPV infection without types 6/11	55 (49.1)	1219 (51.3)	2.16 (0.93-5.02)
PREVALENT HPV INFECTIONS			
Any HPV type	93 (83.0)	1518 (63.9)	3.31 (1.45-7.56)
Non-Oncogenic HPV types only	21 (18.8)	491 (20.7)	2.34 (0.95-5.81)
Oncogenic HPV types only	24 (21.4)	279 (11.8)	4.44 (1.81-10.88)
Both non-oncogenic and oncogenic types	42 (37.5)	386 (16.3)	6.29 (2.67-14.80)
HPV 6/11 ^b	24 (21.4)	101 (4.3)	11.12 (4.54-27.21)
HPV 6/11 only	9 (8.0)	20 (0.8)	16.78 (5.97, 47.19)
HPV 6/11 and other HPV types	15 (13.4)	81 (3.4)	9.55 (3.70-24.63)
HPV infection without types 6/11	69 (61.6)	1417 (59.7)	2.65 (1.15-6.11)

^aReference group for all models.

^bIncludes HPV infections with 6/11 only and infections with 6/11 and other HPV types.

Table 3.3. Multivariable associations for sociodemographic and sexual behavior factors with genital wart incidence after accounting for HPV 6/11 infection.

	Crude HR (95% CI)	Multivariable Entire cohort (N=2487) HR (95% CI) ^c	Multivariable Men with incident HPV (N=1498) HR (95% CI) ^c
Country			
United States	1.00 (ref)	1.00 (ref)	1.00 (ref)
Brazil	0.44 (0.29-0.69)	0.33 (0.20-0.54)	0.32 (0.18-0.56)
Mexico	0.39 (0.24-0.65)	0.45 (0.26-0.77)	0.26 (0.12-0.55)
Age			
18-30	1.00 (ref)	1.00 (ref)	1.00 (ref)
31-44	0.40 (0.25-0.62)	0.44 (0.27-0.71)	0.51 (0.29-0.89)
45-70	0.38 (0.18-0.79)	0.43 (0.20-0.92)	0.28 (0.10-0.82)
Lifetime no. female sexual partners			
0	1.00 (ref)	1.00 (ref)	1.00 (ref)
1	1.63 (0.50-5.34)	1.84 (0.51-6.56)	2.18 (0.45-10.66)
2 to 5	1.72 (0.65-4.58)	2.26 (0.74-6.88)	2.94 (0.72-11.95)
6 to 10	3.45 (1.33-8.94)	4.30 (1.42-12.98)	4.71 (1.19-18.65)
11 to 20	3.29 (1.24-8.73)	4.37 (1.41-13.53)	6.00 (1.51-23.80)
≥21	4.08 (1.55-10.70)	5.69 (1.80-17.97)	7.76 (1.91-31.49)
Refused	3.36 (1.10-10.28)	5.99 (1.73-20.72)	5.19 (1.06-25.27)
p for trend	<0.0001	<0.0001	<0.0001
Condom use during vaginal intercourse in the past three months ^a			
Always	1.00 (ref)	1.00 (ref)	1.00 (ref)
At least half the time	3.26 (1.66-6.44)	2.34 (1.17-4.69)	2.81 (1.13-6.96)
Less than half the time	3.00 (1.45-6.20)	2.44 (1.16-5.14)	2.69 (1.03-7.01)
Never	1.26 (0.62-2.57)	1.31 (0.63-2.71)	1.65 (0.64-4.24)
No vaginal sex in the past three months ^b	1.34 (0.63-2.87)	4.25 (1.17-15.48)	4.70 (0.97-22.92)
Refused	1.97 (0.44-8.89)	0.88 (0.05-16.38)	1.04 (0.02-58.41)
No. male anal sex partners in past three months ^a			
None	1.00 (ref)	1.00 (ref)	1.00 (ref)
1	1.26 (0.75-2.12)	1.10 (0.26-4.70)	1.25 (0.29-5.430)
2	1.88 (0.93-3.79)	3.17 (0.71-14.07)	1.89 (0.24-14.75)
≥3	2.75 (1.47-5.13)	4.53 (1.68-12.20)	2.60 (0.68-9.95)
Refused	1.26 (0.66-2.38)	2.75 (0.71-10.75)	3.40 (0.79-14.59)
No. of times of vaginal intercourse in past three months ^a			
None	1.00 (ref)	1.00 (ref)	1.00 (ref)
1 to 5	1.15 (0.51-2.61)	2.13 (0.58-7.77)	1.57 (0.33-7.38)
6 to 20	1.48 (0.79-2.78)	2.94 (0.90-9.57)	1.70 (0.42-6.97)
≥21	2.28 (1.30-4.01)	4.14 (1.32-13.01)	2.99 (0.77-11.52)
Refused	2.18 (1.03-4.61)	2.63 (0.87-7.96)	2.10 (0.54-8.16)
Ever had a partner with genital warts ^a			
No	1.00 (ref)	1.00 (ref)	1.00 (ref)
Yes	2.81 (1.21-6.48)	2.38 (1.01-5.61)	2.40 (0.84-6.87)
Don't know	2.89 (1.91-4.36)	2.34 (1.51-3.64)	2.50 (1.49-4.19)
Refused	2.48 (0.61-10.16)	1.46 (0.08-26.46)	1.04 (0.02-56.5)

Ever been diagnosed with an STI ^a			
No	1.00 (ref)	1.00 (ref)	1.00 (ref)
Yes	2.52 (1.51-4.20)	1.99 (1.17-3.39)	2.11 (1.15-3.87)
Don't know	0.47 (0.07-3.40)	0.34 (0.05-2.51)	0 (0-.)
Refused	2.02 (0.50-8.20)	1.46 (0.09-23.65)	1.10 (0.02-53.61)

Note: HR - hazard ratio; CI - confidence interval; bolded results have p-values <0.05.

^a Time-dependent covariates.

^b Includes men who only had sex with men in the last three months.

^c Each factor is adjusted for incident HPV 6/11 infection and all other variables in the table.

CHAPTER 4: THIRD MANUSCRIPT: CONCORDANCE OF HUMAN PAPILOMAVIRUS TYPES DETECTED ON THE SURFACE AND IN THE TISSUE OF CONDYLOMA IN MEN

ABSTRACT

The prevalence of HPV in condyloma has often been estimated by sampling the lesion surface, however, HPV types on the lesion surface may not represent the types present in the lesions themselves. We examined the concordance between HPV types detected on the surface and in the tissue of condyloma in men. Samples obtained from the surface of condyloma lesions were both sensitive and specific markers for the presence of any HPV, HPV6 and HPV11 in condyloma tissue. Our results suggest that sampling the surface of condyloma lesions is a non-invasive and accurate marker of the HPV types present in condyloma tissue.

INTRODUCTION

Anogenital condyloma are the most common clinical manifestation of human papillomavirus (HPV) infection [12]. Current standard practice is to diagnose condyloma by visual inspection, and diagnosis is rarely confirmed by biopsy [25]. Thus, clinic-based HPV prevalence studies use samples from the surface of condyloma lesions to estimate the prevalence of HPV genotypes in the lesion tissue [26-28]. It is possible that the HPV types detected on the surface of lesions may not represent the types present in the lesions themselves. Accurate estimates of the distribution of HPV types in condyloma are needed to model the efficacy of the quadrivalent HPV vaccine that protects against

HPV 6, 11, 16 and 18 and for use in the development of future vaccines that protect against additional HPV types.

Since biopsying anogenital condyloma is invasive and may deter individuals from participating in studies that examine the prevalence of HPV types in anogenital lesions, it is important to determine whether sampling the surface of a lesion provides an accurate measure of the HPV types present within it. The purpose of this study was to examine the concordance between HPV types detected on the surface and in the tissue of condyloma and non-condyloma related genital lesions in men.

METHODS

Study participants and sample collection

Participants in this study were enrolled in the prospective HPV in Men (HIM) Study that examined the natural history of HPV infection in men. To be eligible for enrollment, men had to meet the following criteria (a) 18-70 years old; (b) resided in Southern Florida, Sao Paulo, Brazil or the state of Morelos, Mexico; (c) reported no previous diagnosis of penile or anal cancer; (d) reported no prior diagnosis of genital or anal warts; (e) had not participated in an HPV vaccine clinical trial; (f) reported no prior diagnosis of HIV or AIDS; (g) were not currently being treated for an STI; and (h) had not been imprisoned, homeless or in drug treatment in the previous 6 months. A more detailed description of procedures in the HIM Study has been published previously [3, 4, 35].

Men were examined by a trained clinician for the presence of external genital lesions at clinic visits that occurred every six months over four years. Lesions that exhibited clinical features suggestive of condyloma were sampled for the presence of HPV DNA. A pre-wetted Dacron swab was used to sample the surface of the lesion and a biopsy sample was also obtained. Lesions with unknown etiology were also sampled,

although, lesions suspected to be non-HPV related such as Herpes Simplex Virus lesions, pearly penile papules, molluscum contagiosum, skin tags, and sebaceous glands were not sampled or biopsied. Healthy genital skin was also sampled by combining swabs taken from the coronal sulcus/glans penis, penile shaft, and scrotum. Lesions were sampled before the healthy skin to avoid inter-specimen contamination. Patients with lesions suggestive of condyloma or dysplasia underwent removal by shave excision. Excised tissue was placed in 10% buffered formalin and processed at the University of South Florida Dermatopathology Laboratory for pathologic interpretation by the study dermatopathologist, followed by DNA extraction for HPV genotyping.

HPV detection and genotyping

To test for the presence of HPV DNA in biopsied tissue, thin section microtomy specimens were cut from formalin-fixed paraffin-embedded tissue samples. DNA was extracted from the tissue slice, after removal of paraffin by boiling and digestion with proteinase K, followed by precipitation of DNA with isopropanol using the QIAamp DNA FFPE Tissue procedure (Qiagen Inc – USA). The INNO-LiPA HPV genotyping test (Innogenetics) was used to test for the presence of 24 HPV genotypes including 13 oncogenic types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 66) and 11 non-oncogenic types (6, 11, 40, 42, 43, 44, 53, 54, 68, 70, and 74).

DNA was extracted from swab samples of lesions and healthy skin samples using the QIAamp Mini kit following the manufacturer's instructions (Qiagen, Valencia, CA). The polymerase chain reaction consensus primer system PGMY 09/11 was used to amplify a fragment of the HPV L1 gene and test for the presence of HPV DNA. The Linear Array HPV Genotyping Test (Roche Diagnostics, Indianapolis, IN) tested for the presence of 37 HPV genotypes including 13 oncogenic types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 66) and 24 non-oncogenic types (6, 11, 26, 40, 42, 53, 54, 55, 61, 62, 64, 67-73, 81–84, IS39, and CP6108).

A sample was considered HPV positive if HPV DNA was detected by PCR or tested positive for at least one HPV genotype. Beta-globin was detected in 96% (47/49) of biopsies and 92% (36/39) of surface swab specimens. The two biopsy samples negative for β -globin included one condyloma and one benign squamous keratosis lesion. The three swab samples from the surface of lesions negative for β -globin were all benign squamous keratosis lesions. Samples that tested negative for β -globin were not included in analyses.

Statistical analysis

The prevalence of individual HPV types was calculated as the proportion of men who tested positive for each HPV type. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated for individual HPV types to measure the accuracy of sampling the surface of a lesion as a marker of the HPV types present in the lesion tissue. Sensitivity was calculated as the proportion of lesions with biopsy samples positive for a specific HPV type that also had swab samples from the surface of the lesion positive for the same HPV type. Specificity was defined as the proportion of biopsy samples negative for a specific HPV type that also had swab samples negative for the same type. PPV was calculated as the proportion of lesions with swab samples positive for an HPV type that also had a biopsy sample positive for the same HPV type. NPV was the proportion of lesions with swab samples negative for an HPV type that also had a biopsy sample negative for the same HPV type. Exact 95% confidence intervals based on the binomial distribution were calculated for all proportions. Analyses were restricted to the 20 HPV types tested for in both the surface swab and biopsy samples (6, 11, 16, 18, 31, 33, 35, 39, 40, 42, 45, 51-54, 56, 58, 59, 66, and 68).

RESULTS

In total, 34 lesions that tested β -globin positive for samples from both surface swabs and biopsies were included in these analyses. The lesions included 19 histologically confirmed condyloma and 15 lesions that did not demonstrate specific features of condyloma; diagnosis for these lesions was as follows: 13 benign squamous keratosis, one seborrheic keratosis lesion and one lichenoid tissue reaction. The mean ages of men with condyloma and non-condyloma lesions were 28 (range, 18-49) and 29 (range, 18-57), respectively.

Table 1 presents the prevalence of HPV types detected in samples obtained from biopsies, the surface of lesions, and adjacent healthy genital skin for condyloma and non-condyloma lesions. HPV DNA was detected in 95% of tissue samples obtained from condyloma. The individual HPV types detected in the condyloma biopsies were HPV6 (63%), HPV11 (32%), and HPV16 (11%). The prevalence of HPV DNA from swab samples taken from the surface of the condyloma was also 95%. HPV6 (47%), HPV11 (37%), HPV16 (16%), and HPV18 (11%) were the types most commonly detected among condyloma swab samples. Other HPV types including 40, 51, 53, 56, and 59 were detected on the surface of 26% of condyloma. All swab samples taken from healthy genital skin adjacent to condyloma lesions were positive for HPV DNA. Consistent with the biopsy and surface swab samples, HPV6 (42%), HPV11 (37%), and HPV16 (26%) were the most common types detected, however, there was a higher prevalence of other HPV types (47%) than was observed in the tissue or surface swab samples.

HPV DNA was detected in 93% of tissue samples from non-condyloma lesions with HPV6 (47%) and HPV11 (47%) being the most common types detected. Other HPV types including 31, 33, 40, 51, 52, 53, and 58 were present in 20% of non-condyloma biopsies. Swab samples from the surface of non-condyloma lesions had

lower prevalence for any HPV DNA (60%), HPV6 (27%), HPV11 (13%), and HPV16 (7%) than tissue samples.

Table 2 presents the sensitivity, specificity, PPV, and NPV of sampling the surface of lesions and adjacent healthy skin as markers for the HPV types detected in biopsy samples. The sensitivity of swab samples from any type of lesion as a marker for HPV types in biopsied tissue for the corresponding lesion was 78% for any HPV, 63% for HPV6, 62% for HPV11, 50% for HPV16, and 33% for other HPV types. Specificity of swab samples of the lesions was also high for HPV6 (93%), HPV11 (95%), HPV16 (94%), and other HPV types (77%). Samples from the surface of condyloma lesions were more sensitive than swabs from the surface of non-condyloma lesions for any HPV (94% vs. 57%), HPV6 (75% vs. 43%), and HPV11 (100% vs. 29%). Condyloma swabs were less sensitive for HPV16 (50%). Swabs from both condyloma and non-condyloma lesions were highly specific for HPV6 (100% and 88%), HPV11 (92% and 100%), and HPV16 (88% and 100%). Non-condyloma lesion swabs were not sensitive (33%), but were highly specific (83%) as markers of other HPV types in lesion tissue. Similar to samples from the surface of lesions, swabs from adjacent healthy genital skin were more sensitive for HPV types in condyloma tissue than non-condyloma tissue.

Among condyloma lesions there was high PPV for swabbing the surface of the lesion for any HPV (94%), HPV6 (100%), and HPV11 (86%). High PPV signifies a low rate of false positives for specific HPV types within the condyloma tissue when swabbing the surface of the lesion. The frequency of false negatives was also low as indicated by high NPV for HPV6 (70%) and HPV11 (100%).

When different lesion types (i.e., condyloma vs. non-condyloma) were classified by visual inspection alone and not pathologically confirmed, 33 of the 34 lesions were diagnosed as condyloma. Based on visual inspection, the sensitivity of swabbing the surface of the lesion for any HPV (81%) and HPV type 6 (63%) and 11 (67%) was lower

than among pathologically confirmed condyloma. Specificity of HPV types 6 (93%) and 11 (95%) however remained high among the condyloma diagnosed by visual inspection.

DISCUSSION

To our knowledge, this is the first study to examine the concordance between HPV types detected on the surface and in the tissue of condyloma and non-condyloma genital lesions in men. Swabs from the surface of condyloma were highly sensitive and specific as markers for HPV types present in condyloma tissue. However, non-condyloma lesions were less sensitive markers of HPV types in lesion tissue and may potentially underestimate the prevalence of individual HPV types within the lesion.

HPV DNA was detected in 95% of condyloma tissue samples from the current study. This is consistent with previous studies that biopsied condyloma and reported HPV prevalence estimates of 91% among women in the placebo arm of a vaccine trial [24] and 100% of men and women seeking treatment in a standard clinic setting [33]. Likewise, HPV prevalence estimates ranged from 95% to 100% in studies that tested samples from swabbing the surface of the condyloma [26-28]. Also similar to our results, previous studies detected HPV 6/11 in 86% to 100% of condyloma [24, 26-28, 33]. The finding that HPV16 was the third most common type detected in condyloma biopsies after HPV6 and HPV11 is consistent with previous studies that also reported a high ($\geq 25\%$) prevalence of HPV16 [24, 33]. Among condyloma lesions, the prevalence of any HPV and types 6 and 11 was higher among the biopsy samples than samples from the surface of the lesion. The sensitivities of the assays used to test the different sample types may account for some of this difference. The INNO-LiPA assay used to test the biopsy samples is more sensitive for detecting HPV than the Linear Array assay used to test swabs from the surface of the lesion [44, 45], therefore prevalence of HPV is more likely to be higher for biopsy samples.

In this study, samples obtained from the surface of histologically confirmed condyloma were sensitive markers for the presence of HPV in condyloma tissue. However, in this study a significant number of lesions diagnosed as condyloma by visual inspection were not confirmed histologically. In the current study, visual inspection was 94% sensitive and 23% specific for identifying histologically confirmed condyloma. This apparent low specificity, however, likely relates to the difficulty of histologic diagnosis of early condyloma, since the non-condyloma lesions had the same rate of HPV detection by PCR as the condyloma lesions. A significant number of these non-condyloma (13/15, 87%) were given the diagnosis of “benign squamous keratosis,” a relatively non-specific pathologic diagnosis for lesions that show some but not all of the diagnostic criteria for HPV infection. It is compelling that swabs of these non-condyloma lesions were found to be less sensitive as markers for the presence of HPV than the lesion tissue, a phenomenon which may relate to low viral load of these pathologically subtle lesions. Testing samples from the surface of condyloma diagnosed based on visual inspection alone may slightly underestimate the prevalence of HPV genotypes in condyloma.

Small sample size is a limitation in our analyses and may have contributed to the low sensitivity observed for some HPV types. Samples from the surface of condyloma were only 50% sensitive to the presence of HPV16 in condyloma tissue. Since only two condyloma biopsies tested positive for HPV16, a larger sample size with a correspondingly higher prevalence of HPV16 might provide a more accurate measure of sensitivity for this genotype. Given that most HPV genotypes were present in less than 10% of lesions, we were not able to assess concordance for individual HPV types other than 6, 11, and 16. Future studies should confirm our findings using a larger sample size.

In summary, our results suggest that sampling the surface of a condyloma lesion is a non-invasive and accurate marker of HPV types present in condyloma tissue.

Therefore, it may not be necessary to add an invasive biopsy procedure to research protocols for studying condyloma in a standard clinic setting.

Table4.1. Prevalence of HPV genotypes in samples from biopsies, the surface of lesions, and adjacent healthy genital skin for condyloma and non-condyloma genital lesions.

HPV genotype	Biopsy samples			Samples from the surface of the lesion			Samples from adjacent healthy skin		
	All lesions (N=34)	Condyloma (N=19)	Non-condyloma (N=15)	All lesions (N=34)	Condyloma (N=19)	Non-condyloma (N=15)	All lesions (N=34)	Condyloma (N=19)	Non-condyloma (N=15)
Any HPV type	32 (94%)	18 (95%)	14 (93%)	27 (79%)	18 (95%)	9 (60%)	31 (91%)	19 (100%)	12 (80%)
6	19 (56%)	12 (63%)	7 (47%)	13 (38%)	9 (47%)	4 (27%)	12 (35%)	8 (42%)	4 (27%)
11	13 (38%)	6 (32%)	7 (47%)	9 (26%)	7 (37%)	2 (13%)	10 (29%)	7 (37%)	3 (20%)
16	2 (6%)	2 (11%)	0 (0%)	3 (9%)	3 (16%)	1 (7%)	6 (18%)	5 (26%)	1 (7%)
18	0 (0%)	0 (0%)	0 (0%)	3 (9%)	2 (11%)	1 (7%)	4 (12%)	2 (11%)	2 (13%)
Other HPV types ^a	3 (9%)	0 (0%)	3 (20%)	8 (24%)	5 (26%)	3 (20%)	16 (47%)	9 (47%)	7 (47%)

^aIncludes HPV 31, 33, 39, 40, 42, 51, 52, 53, 54, 56, 58, 59, and 66.

Table 4.2. Sensitivity, specificity, negative predictive value, and positive predictive value of samples from the surface of lesions and adjacent healthy skin as markers of HPV types present in biopsy samples in condyloma and non-condyloma genital lesions.

	SWABS OF THE SURFACE OF THE LESION			SWABS OF ADJACENT HEALTHY SKIN		
	All lesions (95% CI)	Condyloma (95% CI)	Non- condyloma (95% CI)	All lesions (95% CI)	Condyloma (95% CI)	Non- condyloma (95% CI)
Any HPV						
Sensitivity	78 (60-91)	94 (73-100)	57 (29-82)	91 (75-98)	100 (81-100)	79 (49-95)
Specificity	n/a ^a	n/a ^b	n/a ^a	n/a ^a	n/a ^a	n/a ^a
PPV	93 (76-99)	94 (73-100)	89 (52-100)	94 (79-99)	95 (74-100)	92 (62-100)
NPV	100 (59-100)	n/a ^b	100 (54-100)	93 (75-99)	95 (74-100)	92 (62-100)
HPV 6						
Sensitivity	63 (38-84)	75 (43-95)	43 (10-82)	58 (34-80)	67 (35-90)	43 (10-82)
Specificity	93 (68-100)	100 (59-100)	88 (47-100)	93 (68-100)	100 (59-100)	88 (47-100)
PPV	92 (64-100)	100 (66-100)	75 (19-99)	92 (62-100)	100 (66-100)	75 (19-99)
NPV	67 (43-85)	70 (35-93)	64 (31-89)	67 (43-85)	64 (31-89)	64 (31-89)
HPV 11						
Sensitivity	62 (32-86)	100 (54-100)	29 (4-71)	69 (39-91)	100 (54-100)	43 (10-82)
Specificity	95 (76-100)	92 (64-100)	100 (63-100)	95 (76-100)	92 (64-100)	100 (63-100)
PPV	89 (52-100)	86 (42-100)	100 (16-100)	90 (56-100)	86 (42-100)	100 (16-100)
NPV	80 (59-93)	100 (74-100)	62 (32-86)	80 (59-93)	100 (74-100)	67 (35-90)
HPV 16						
Sensitivity	50 (1-99)	50 (1-99)	n/a ^c	100 (16-100)	100 (16-100)	n/a ^c
Specificity	94 (79-100)	88 (64-99)	100 (78-100)	88 (71-96)	82 (57-96)	93 (68-100)
PPV	33 (1-91)	33 (1-91)	n/a ^c	33 (4-78)	33 (1-91)	n/a ^c
NPV	97 (83-100)	94 (70-100)	100 (78-100)	97 (83-100)	100 (77-100)	100 (77-100)
HPV Other^e						
Sensitivity	33 (9-91)	n/a ^d	33 (1-91)	33 (1-91)	n/a ^d	33 (1-91)
Specificity	77 (59-90)	74 (49-91)	83 (52-98)	52 (33-70)	53 (29-76)	50 (21-79)
PPV	13 (0-53)	n/a ^d	33 (1-91)	6 (0-30)	n/a ^d	33 (1-91)
NPV	92 (75-99)	100 (77-100)	83 (52-98)	92 (75-99)	100 (69-100)	75 (35-97)

Note: CI - confidence interval; PPV – positive predictive value; NPV – negative predictive value.

^aNot calculated due to small numbers.

^bNot calculated because no men with condyloma had skin swabs negative for HPV.

^cNot calculated because HPV16 was not detected in any non-condyloma biopsies.

^dNot calculated because other HPV types were not detected in any condyloma biopsies.

^eIncludes HPV 31, 33, 39, 40, 42, 51, 52, 53, 54, 56, 58, 59, and 66.

CHAPTER 5: CONCLUSIONS AND FUTURE DIRECTIONS

SUMMARY OF FINDINGS

This study examined the natural history of HPV related condyloma in a cohort of 2,487 men ages 18-70 from the US, Brazil, and Mexico enrolled in the prospective HIM Study. This is one of the first studies to examine the progression from HPV infection to condyloma development and prospectively assess the sexual behavioral factors associated with condyloma in men.

The probability of developing a condyloma over 24-months was 5.2% and the median time to condyloma detection was 17.1 months. HPV 6 and 11 were the HPV types most strongly associated with development of condyloma. Men who had an incident HPV 6/11 infection had 12 times the risk of developing condyloma compared to men who were HPV negative. Men with incident HPV 6/11 infections also had the highest probability of developing condyloma in a 24-month period (14.6%), and had the shortest time to detection of condyloma (median of 6.2 months).

The surfaces of condyloma lesions were sampled to estimate the prevalence of HPV types in the condyloma. HPV types 6, 11, and 16 were the most common types detected, however, the HPV types detected on the surface of lesions may not represent the HPV types present in the lesions themselves. Nineteen pathologically confirmed condyloma with samples obtained from the surface of the lesion and biopsied tissue were genotyped to assess the concordance between HPV types detected on the surface and in the tissue of condyloma lesions. Sampling the surface of condyloma was both highly sensitive and specific as a marker for the HPV types present in the condyloma

tissue. Therefore, HPV prevalence estimates based on sampling the surface of lesions are likely accurate estimate of the HPV types present in lesion tissue.

Recent sexual behavior was strongly associated with risk of developing condyloma. Having three or more male anal sex partners in the previous three months was associated with an almost five times greater risk of condyloma compared to men with no male sexual partners. Frequent vaginal intercourse and infrequent condom use during vaginal intercourse in the recent past also significantly increased the risk for condyloma. Other factors associated with condyloma incidence were a high number of lifetime female partners, having a partner with condyloma, and ever being diagnosed with an STI. Younger age was also a significant risk factor; men who were older than age 30 had a 50% reduced risk of condyloma compared to men ages 18-30.

Multicollinearity could be present in multivariable regression models if two or more of the predictor variables in the model are highly correlated. A high amount of multicollinearity would result in increased standard errors of the beta estimates and subsequent wide confidence intervals. As a result, variables that are actually significant predictors would appear to have no significant association with the outcome. The Pearson correlation coefficient values between pairs of the variables included in the final multivariable model (age, country, lifetime number of female partners, condom use, recent number of male sexual partners, frequency of vaginal intercourse, having a partner with condyloma, and having an STI) ranged from -0.02 to 0.21. Given that no two variables in the model were highly correlated, it is not likely that a high degree of multicollinearity was present in the final multivariable model examining the factors associated with incident condyloma.

Eight hazard regression models were run using the same dataset to examine the association between HPV infection with different HPV types and risk of condyloma. The more HPV types that are examined in relation to condyloma risk, the greater the

likelihood that there will be a significant association observed simply by chance (i.e., type 1 error) due to multiple comparisons. Multiple testing correction methods, such as Bonferroni, require that a p-value smaller than 0.05 be observed to declare an association is significant. Though correction for multiple comparisons was not included in the models, a type 1 error is not likely given that the HRs for these models were very high and the corresponding p-values were often <0.0001.

This study has limitations that need to be considered when interpreting the results. Condyloma were identified by visual inspection and biopsy samples were not obtained to confirm that lesions were true condyloma. Preliminary data from biopsy results collected in the HIM Study showed that compared to the gold standard of diagnosing condyloma by pathologic review, diagnosing condyloma by visual inspection was highly sensitive (94%), but not very specific (23%). The low specificity suggests that some of the condyloma included in these analyses were actually non-HPV related skin conditions that resembled condyloma, such as benign squamous keratosis or seborrheic keratosis. Despite the potential misclassification of diagnosing condyloma by visual inspection, most previous studies also did not have pathologically confirmation that lesions were condyloma. Another limitation is the generalizability of the findings is likely limited due to the self-selection of participants. Men who agree to participate in a four year prospective study may not be representative of the underlying population from each country. However, our results are likely more generalizable than previous studies that were based on populations of men who have sex with men or men who were seeking treatment for an STI.

Despite limitations, there are strengths of the study to note as well. An extensive risk factor questionnaire was used to collect data on multiple lifetime and recent sexual behaviors. The prospective study design allowed for measuring changes in sexual behavior over time. Repeated measures of behavior were important given that recent

sexual behavior was strongly associated with risk of condyloma. The prospective study design also allowed collection of data on sexual behavior before men developed condyloma. Previous studies of risk factors for condyloma in men were case-control studies that collected risk factor data after men were diagnosed with condyloma. This could bias results if being diagnosed with condyloma caused men to alter their sexual behavior (e.g., use condoms more frequently) or affected how accurately they recalled their sexual habits. By collecting data on lifetime and recent sexual behavior before men were diagnosed with condyloma, we were able to minimize the chance of recall bias.

FUTURE RESEARCH

This study was one of the first to examine incidence and risk factors associated with condyloma in a prospective study largely including men from the general community, however, there are limitations that need to be addressed in future studies. The major limitation was identification of condyloma by visual inspection. Future studies should biopsy suspected condyloma lesions to histologically confirm a lesion is condyloma and thereby minimize misclassification. Condyloma incidence, time from HPV infection to condyloma development, and risk factors for condyloma should then be examined among histologically confirmed condyloma to assess if the results are consistent with studies that relied on diagnosis by visual inspection.

Unclassified infections that tested positive for HPV DNA, but not for any specific HPV genotypes, were observed in 5% of condyloma in this study. Cutaneous HPV types may account for some of the unclassified infections observed, however, no studies to date have utilized assays that test for the presence of cutaneous HPV in condyloma samples. Futures studies should test for cutaneous in addition to mucosal HPV types in an effort to identify the unclassified genotypes present in condyloma.

The cohort used to examine risk factors for condyloma in this study included heterosexual men and men who have sex with men. The placebo arm of an HPV vaccine clinical trial of men observed a significantly higher incidence of condyloma in men who have sex with men compared to men with only female sexual partners [14]. Future studies should assess incidence of condyloma stratified by sexual orientation and examine whether the risk factors for condyloma differ between men who have sex with men and men who have sex with women only. Differences in risk factors for condyloma by sexual orientation may warrant different prevention strategies that focus on modifying behavior in addition to vaccination.

Though condyloma are the most common clinical manifestation of anogenital HPV infection, penile intraepithelial neoplasia (PIN), the pre-cursor lesion to approximately 50% of invasive penile carcinomas, is another HPV related external genital lesion that has not been studied extensively. Since these lesions have the potential to develop into invasive cancer, it is important to identify risk factors associated with incidence of these lesions to create appropriate prevention strategies. Accurate estimates of the prevalence of HPV types in PIN lesions is also needed to model the efficacy of the current HPV vaccine that protect against oncogenic HPV types 16 and 18.

Another area that needs further study is the role of HPV antibodies in protecting against development of condyloma. Using archived serum samples from HIM Study participants, a future study should examine whether HPV antibodies reduce the risk of condyloma by comparing condyloma incidence between men who are sero-negative and sero-positive for different HPV types.

CHAPTER 6: REFERENCES

1. Cates W, Jr. Estimates of the incidence and prevalence of sexually transmitted diseases in the United States. American Social Health Association Panel. *Sex Transm Dis* 1999;26:S2-7.
2. Human papillomaviruses. 2007;IARC Monogr Eval Carcinog Risk Hum:1-636.
3. Giuliano AR, Lee J, Fulp W, et al. The Incidence and Clearance of Genital Human Papillomavirus Infection in Men: The HIM Study. *Lancet* 2011 (in press).
4. Giuliano AR, Lazcano-Ponce E, Villa LL, et al. The human papillomavirus infection in men study: human papillomavirus prevalence and type distribution among men residing in Brazil, Mexico, and the United States. *Cancer Epidemiol Biomarkers Prev* 2008;17:2036-43.
5. Giuliano AR, Lu B, Nielson CM, et al. Age-specific prevalence, incidence, and duration of human papillomavirus infections in a cohort of 290 US men. *J Infect Dis* 2008;198:827-3.
6. Partridge JM, Hughes JP, Feng Q, et al. Genital human papillomavirus infection in men: incidence and risk factors in a cohort of university students. *J Infect Dis* 2007;196:1128-36.
7. Dunne EF, Nielson CM, Stone KM, Markowitz LE and Giuliano AR. Prevalence of HPV infection among men: A systematic review of the literature. *J Infect Dis* 2006;194:1044-57.
8. Scheurer ME, Tortolero-Luna G and Adler-Storthz K. Human papillomavirus infection: biology, epidemiology, and prevention. *Int J Gynecol Cancer* 2005;15:727-46.
9. Jeynes C, Chung MC and Challenor R. 'Shame on you' - the psychosocial impact of genital warts. *Int J STD AIDS* 2009;20:557-60.
10. Woodhall S, Ramsey T, Cai C, et al. Estimation of the impact of genital warts on health-related quality of life. *Sex Transm Infect* 2008;84:161-6.
11. Oriel JD. Natural history of genital warts. *Br J Vener Dis* 1971;47:1-13.
12. Lacey CJ, Lowndes CM and Shah KV. Chapter 4: Burden and management of non-cancerous HPV-related conditions: HPV-6/11 disease. *Vaccine* 2006;24 Suppl 3:S3/35-41.
13. Insinga RP, Dasbach EJ and Elbasha EH. Assessing the annual economic burden of preventing and treating anogenital human papillomavirus-related disease in the US: analytic framework and review of the literature. *Pharmacoeconomics* 2005;23:1107-22.

14. Giuliano AR, Palefsky JM, Goldstone S, et al. Efficacy of quadrivalent HPV vaccine against HPV Infection and disease in males. *N Engl J Med* 2011;364:401-11.
15. Hoy T, Singhal PK, Willey VJ and Insinga RP. Assessing incidence and economic burden of genital warts with data from a US commercially insured population. *Curr Med Res Opin* 2009;25:2343-51.
16. Insinga RP, Dasbach EJ and Myers ER. The health and economic burden of genital warts in a set of private health plans in the United States. *Clin Infect Dis* 2003;36:1397-403.
17. Koshiol JE, Laurent SA and Pimenta JM. Rate and predictors of new genital warts claims and genital warts-related healthcare utilization among privately insured patients in the United States. *Sex Transm Dis* 2004;31:748-52.
18. Arima Y, Winer RL, Feng Q, et al. Development of genital warts after incident detection of human papillomavirus infection in young men. *J Infect Dis* 2010;202:1181-4.
19. Van Den Eeden SK, Habel LA, Sherman KJ, McKnight B, Stergachis A and Daling JR. Risk factors for incident and recurrent condylomata acuminata among men. A population-based study. *Sex Transm Dis* 1998;25:278-84.
20. Wen LM, Estcourt CS, Simpson JM and Mindel A. Risk factors for the acquisition of genital warts: are condoms protective? *Sex Transm Infect* 1999;75:312-6.
21. Hughes G, Catchpole M, Rogers PA, et al. Comparison of risk factors for four sexually transmitted infections: results from a study of attenders at three genitourinary medicine clinics in England. *Sex Transm Infect* 2000;76:262-7.
22. Jin F, Prestage GP, Kippax SC, et al. Risk factors for genital and anal warts in a prospective cohort of HIV-negative homosexual men: the HIM study. *Sex Transm Dis* 2007;34:488-93.
23. Winer RL, Kiviat NB, Hughes JP, et al. Development and duration of human papillomavirus lesions, after initial infection. *J Infect Dis* 2005;191:731-8.
24. Garland SM, Steben M, Sings HL, et al. Natural history of genital warts: analysis of the placebo arm of 2 randomized phase III trials of a quadrivalent human papillomavirus (types 6, 11, 16, and 18) vaccine. *J Infect Dis* 2009;199:805-14.
25. Wiley DJ, Douglas J, Beutner K, et al. External genital warts: diagnosis, treatment, and prevention. *Clin Infect Dis* 2002;35:S210-24.
26. Chan PK, Luk AC, Luk TN, et al. Distribution of human papillomavirus types in anogenital warts of men. *J Clin Virol* 2009;44:111-4.
27. Aubin F, Pretet JL, Jacquard AC, et al. Human papillomavirus genotype distribution in external acuminata condylomata: a Large French National Study (EDiTH IV). *Clin Infect Dis* 2008;47:610-5.

28. Greer CE, Wheeler CM, Ladner MB, et al. Human papillomavirus (HPV) type distribution and serological response to HPV type 6 virus-like particles in patients with genital warts. *J Clin Microbiol* 1995;33:2058-63.
29. Dinh TH, Sternberg M, Dunne EF and Markowitz LE. Genital warts among 18- to 59-year-olds in the United States, national health and nutrition examination survey, 1999--2004. *Sex Transm Dis* 2008;35:357-60.
30. Koutsky L. Epidemiology of genital human papillomavirus infection. *Am J Med* 1997;102:3-8.
31. Lacey CJ. Therapy for genital human papillomavirus-related disease. *J Clin Virol* 2005;32 Suppl 1:S82-90.
32. Giuliano AR, Palefsky JM, Goldstone S, et al. Quadrivalent HPV vaccine efficacy against HPV 6/11/16/18 infection and disease in men. *N Engl J Med* 2011 (in press).
33. Brown DR, Schroeder JM, Bryan JT, Stoler MH and Fife KH. Detection of multiple human papillomavirus types in Condylomata acuminata lesions from otherwise healthy and immunosuppressed patients. *J Clin Microbiol* 1999;37:3316-22.
34. Arima Y, Winer RL, Feng Q, et al. Development of genital warts after incident detection of human papillomavirus infection in young men. *J Infect Dis*;202:1181-4.
35. Giuliano AR, Lazcano E, Villa LL, et al. Circumcision and sexual behavior: factors independently associated with human papillomavirus detection among men in the HIM study. *Int J Cancer* 2009;124:1251-7.
36. Ulm K. A simple method to calculate the confidence interval of a standardized mortality ratio (SMR). *Am J Epidemiol* 1990;131:373-5.
37. Anic GM, Messina JL, Stoler MH, et al. Concordance of human papillomavirus types detected on the surface and in the tissue of condyloma in men. *JID* (under review).
38. Garrido-Rios AA, Sanz-Munoz C, Miranda-Sivelo A and Miranda-Romero A. Major depressive episode secondary to condylomata acuminata. *Gen Hosp Psychiatry* 2010;32:446 e3-5.
39. Nielson CM, Harris RB, Dunne EF, et al. Risk factors for anogenital human papillomavirus infection in men. *J Infect Dis* 2007;196:1137-45.
40. Lu B, Wu Y, Nielson CM, et al. Factors associated with acquisition and clearance of human papillomavirus infection in a cohort of US men: a prospective study. *J Infect Dis* 2009;199:362-71.
41. Giuliano AR, Tortolero-Luna G, Ferrer E, et al. Epidemiology of human papillomavirus infection in men, cancers other than cervical and benign conditions. *Vaccine* 2008;26 Suppl 10:K17-28.

42. Bergman A, Nalick R. Prevalence of human papillomavirus infection in men. Comparison of the partners of infected and uninfected women. *J Reprod Med* 1992;37:710-2.
43. Markowitz LE, Sternberg M, Dunne EF, McQuillan G and Unger ER. Seroprevalence of human papillomavirus types 6, 11, 16, and 18 in the United States: National Health and Nutrition Examination Survey 2003-2004. *J Infect Dis* 2009;200:1059-67.
44. Shanesmith R, Allen RA, Moore WE, et al. Comparison of 2 line blot assays for defining HPV genotypes in oral and oropharyngeal squamous cell carcinomas. *Diagn Microbiol Infect Dis* 2011.
45. Tan SE, Garland SM, Rumbold AR and Tabrizi SN. Human papillomavirus genotyping using archival vulval dysplastic or neoplastic biopsy tissues: comparison between the INNO-LiPA and linear array assays. *J Clin Microbiol* 2010;48:1458-60.

APPENDIX

APPENDIX A: LITERATURE REVIEW

Human Papillomavirus

Human papillomavirus (HPV) is the most common sexually transmitted infection in the United States, with an estimated 6.2 million new cases each year [1]. HPV is an established cause of cervical cancer and its precursor lesion, cervical intraepithelial neoplasia (CIN). The virus is also known to be associated with cancers at other sites including the oropharynx, anus, penis, vulva and vagina [2]. HPV related external genital lesions (EGL) in men include genital warts and penile intraepithelial neoplasia (PIN), a precursor lesion to some penile carcinomas.

Over 100 HPV types have been identified and approximately 40 of these types infect the anogenital region. HPV types are classified as non-oncogenic types (e.g. 6 and 11) that are associated with benign conditions such as genital warts, and oncogenic types (e.g. 16, 18, 45, and 31) that are associated with intraepithelial neoplasia and invasive carcinoma. The majority of HPV infections are asymptomatic and 70% of infections with clear within 12 months, however persistent infections can progress to dysplasia or invasive carcinoma [3].

The quadrivalent vaccine Gardasil, that protects against HPV types 6, 11, 16, and 18, is currently available to women, and is effective at reducing the incidence of genital warts and precancerous lesions of the cervix, vulva and vagina [4]. In October 2009, the U.S. Food and Drug Administration approved the use of Gardasil in men ages 9 to 26. The vaccine is currently being tested among men and has shown to be effective at reducing the incidence of HPV infection and genital warts in men ages 16-26 [5]. Vaccinating men may also reduce the incidence of HPV related penile dysplasia and penile carcinoma. More data on HPV type distribution of male genital lesions and time from HPV infection to lesion development are needed to assess the potential impact of a

prophylactic vaccine in men, and to develop future vaccines that protect against additional HPV types.

To date, most research on the progression of HPV infection to disease has focused on women. Little is known about the natural history of HPV related disease in men, including the HPV types associated with lesions, the rate of progression from HPV infection to lesion development, the proportion of type-specific HPV infections that progress to lesions and the sociodemographic and sexual behavior factors associated with the development of lesions. Since male genital lesions are reservoirs for HPV infection, understanding the natural history of HPV related genital disease in men has the potential to not only reduce the burden of male disease, but also reduce the rate of HPV transmission to women.

Genital HPV Infection in Men

A recent systematic review reported the prevalence of genital HPV DNA among men ranging from 1.3%-72.9% (with most studies reporting $\geq 20\%$) [6]. HPV prevalence varies widely across studies due to differences in the populations studied, genital sites sampled (e.g., scrotum, shaft, glans, etc.), and HPV DNA detection methods used. Prevalence tends to be higher in studies that use more sensitive DNA detection methods or sample for HPV DNA at multiple sites on the genitalia. There are also differences across studies in the number of HPV types tested for. Some studies only tested for HPV types 16 and 18, while other studies utilized assays that could detect more than 30 HPV types.

The multi-national HIM study (the source of the lesion data for this study) reported an overall HPV prevalence of 65.1% with a statistically significant higher prevalence in Brazil (72.3%) than the US (61.3%) or Mexico (61.9%) [7]. The HIM study prevalence estimate is based on sampling from four anatomic sites (coronal sulcus, glans, shaft, and scrotum) and testing for the presence of 37 types of HPV. Among

men with HPV infections, 12.0% had oncogenic types only, 20.7% had non-oncogenic types only, 17.8% had both oncogenic and non-oncogenic types, and 14.7% had unclassified types only (tested positive for HPV by PCR, but negative for all of the 37 mucosal HPV types tested for). There was also a high rate of multiple infections (25.7%). HPV 6 (6.6%) and HPV 16 (6.5%) were among the most common types, while HPV 11 (1.5%) and HPV 18 (1.7%) were detected less frequently. Similar results were seen in a cross-sectional study of US men that examined HPV prevalence at six anogenital sites (glans/corona, penile shaft, scrotum, urethra, perianal area and anal canal) [8]. HPV was detected in 65.5% of men, with half the men (51.2%) testing positive for a known oncogenic or non-oncogenic type and another 14.3% testing positive for an unclassified HPV type. HPV 16 was the most common type detected (11.4%).

Only a few cohort studies have examined the incidence and duration of HPV infection in men [9-14]. It is estimated that the cumulative incidence of male HPV infection over a 12-month period is between 29% and 39% [13, 14]. The majority of infections clear in less than 12 months, with one study of US men reporting a median time to clearance of 5.9 months [15].

The factors independently associated with HPV infection in men include not being circumcised [12, 15-18], lack of condom use [19-21], a history of having ever smoked [17, 19, 21], and a high number of lifetime sexual partners [15, 16, 19-21]. Smoking impairs the humoral immune response (i.e., development of antibodies) to HPV infection [22]. An impaired immune response can increase the risk of becoming infected after being exposed to HPV or increase the likelihood of developing a persistent HPV infection. Being circumcised may reduce the risk of infection by increasing the amount of keratinized epithelium present, which provides a more protective barrier than mucosal tissue against HPV infection. Men who are not circumcised have a greater risk of micro

tears or abrasions (portals of entry for HPV) when the mucosal lining of the foreskin is exposed during intercourse [23].

There does not appear to be an association between age and HPV prevalence in men. Studies consistently show that prevalence of HPV infection in men remains constant over the lifetime [6, 15, 19]. This is in contrast to the pattern observed in women, where HPV prevalence is highest among women 18-24 and then decreases until middle age, after which it remains steady for the remainder of the lifespan [24].

Epidemiology of Genital Warts

Genital warts are a common sexually transmitted disease in the US, with an estimated 1 million new cases each year [25]. Data from private health plans estimate that the prevalence of genital warts is highest among men ages 25-29 and decreases with age [26]. Though there are no national data on the prevalence of genital warts in the US, in the 1999-2004 National Health and Nutrition Examination Survey, 5.6% of sexually active adults ages 18-59 reported having ever been diagnosed with genital warts (7.2% women and 4.0% men) [27]. There are no precise estimates of genital wart incidence among men in the US.

Though genital warts are benign and not associated with mortality, they are a source of psychosocial distress, such as shame and embarrassment [28]. On rare occasions they can develop into malignant conditions such as Buschke–Lowenstein tumors or penile carcinoma [29]. Approximately 20-30% of genital warts will spontaneously regress [30], however recurrence of warts is common, resulting in high medical costs for repeated treatment. An estimated \$200 million is spent annually in the US for direct medical costs of genital wart treatment [31].

The incubation period is 3 weeks to 8 months, with most warts developing 2-3 months after infection with HPV [32]. Genital warts are highly infectious and approximately 65% of people whose sexual partner has genital warts will develop warts

themselves [32]. Circumcised men are more likely to report a history of genital warts (4.5%) than uncircumcised men (2.4%) [27]. This is opposite from the association observed with HPV infection, where circumcised men are less likely to have HPV. A possible explanation is that the uncircumcised men failed to detect genital warts that developed under the foreskin. Among sexually active adults in the US, people who had >10 sexual partners in their lifetime had more than 7 times the odds of reporting a history of genital warts compared to adults with only 1 or 2 lifetime partners (OR=7.6; 95% CI: 4.1-13.9) [27].

HPV and Genital Warts

More than 90% of genital warts are caused by non-oncogenic HPV types 6 and 11 [29]. Data available for the HPV type distribution of genital warts in men are sparse. The largest case series to look at a broad range of HPV types in male genital warts included 135 men from Hong Kong [33]. HPV DNA was detected in 96% of the warts. Among HPV positive warts, 75.4% had non-oncogenic types only, 3.8% had oncogenic types only, and 20.8% had both oncogenic and non-oncogenic types. There was a high rate of multiple infections (33.8%), often including coinfection with oncogenic types. HPV 6 was the most common type detected (54.6%), followed by HPV 11 (40.8%) and HPV 16 (6.2%). A smaller case series of 12 men detected HPV in 100% of genital warts. Again, the most common HPV types were HPV 6 (75.0%), HPV 11 (16.7%) and HPV 16 (8.3%) [34].

HPV 6/11 have consistently been the most common types detected in genital warts. Previous studies reported the HPV 6/11 prevalence in genital warts to be 86% among young women in the placebo arm of an HPV vaccine trial [35], 89% in men from Hong Kong [33], 90% among French men ages 18-72 [36], and greater than 95% in two small US studies that included fewer than 50 men [37, 38]. Oncogenic HPV types have

also commonly been detected in genital warts and several studies reported a high prevalence of HPV 16 [33, 35, 36, 38].

REFERENCES

1. Cates W, Jr. Estimates of the incidence and prevalence of sexually transmitted diseases in the United States. American Social Health Association Panel. *Sex Transm Dis* 1999;26:S2-7.
2. Human papillomaviruses. 2007;IARC Monogr Eval Carcinog Risk Hum:1-636.
3. Scheurer ME, Tortolero-Luna G and Adler-Storthz K. Human papillomavirus infection: biology, epidemiology, and prevention. *Int J Gynecol Cancer* 2005;15:727-46.
4. Koutsky LA, Harper DM. Chapter 13: Current findings from prophylactic HPV vaccine trials. *Vaccine* 2006;24 Suppl 3:114-21.
5. Giuliano AR, Palefsky JM, Goldstone S, et al. Quadrivalent HPV vaccine efficacy against HPV 6/11/16/18 infection and disease in men. *N Engl J Med* 2011 (in press).
6. Dunne EF, Nielson CM, Stone KM, Markowitz LE and Giuliano AR. Prevalence of HPV infection among men: A systematic review of the literature. *J Infect Dis* 2006;194:1044-57.
7. Giuliano AR, Lazcano-Ponce E, Villa LL, et al. The human papillomavirus infection in men study: human papillomavirus prevalence and type distribution among men residing in Brazil, Mexico, and the United States. *Cancer Epidemiol Biomarkers Prev* 2008;17:2036-43.
8. Nielson CM, Flores R, Harris RB, et al. Human papillomavirus prevalence and type distribution in male anogenital sites and semen. *Cancer Epidemiol Biomarkers Prev* 2007;16:1107-14.
9. Wikstrom A, Popescu C and Forslund O. Asymptomatic penile HPV infection: a prospective study. *Int J STD AIDS* 2000;11:80-4.
10. Van Doornum GJ, Prins M, Juffermans LH, et al. Regional distribution and incidence of human papillomavirus infections among heterosexual men and women with multiple sexual partners: a prospective study. *Genitourin Med* 1994;70:240-6.
11. Kjaer SK, Munk C, Winther JF, Jorgensen HO, Meijer CJ and van den Brule AJ. Acquisition and persistence of human papillomavirus infection in younger men: a prospective follow-up study among Danish soldiers. *Cancer Epidemiol Biomarkers Prev* 2005;14:1528-33.
12. Lajous M, Mueller N, Cruz-Valdez A, et al. Determinants of prevalence, acquisition, and persistence of human papillomavirus in healthy Mexican military men. *Cancer Epidemiol Biomarkers Prev* 2005;14:1710-6.
13. Partridge JM, Hughes JP, Feng Q, et al. Genital human papillomavirus infection in men: incidence and risk factors in a cohort of university students. *J Infect Dis* 2007;196:1128-36.

14. Giuliano AR, Lu B, Nielson CM, et al. Age-specific prevalence, incidence, and duration of human papillomavirus infections in a cohort of 290 US men. *J Infect Dis* 2008;198:827-35.
15. Lu B, Wu Y, Nielson CM, et al. Factors associated with acquisition and clearance of human papillomavirus infection in a cohort of US men: a prospective study. *J Infect Dis* 2009;199:362-71.
16. Giuliano AR, Lazcano E, Villa LL, et al. Circumcision and sexual behavior: factors independently associated with human papillomavirus detection among men in the HIM study. *Int J Cancer* 2009;124:1251-7.
17. Vaccarella S, Lazcano-Ponce E, Castro-Garduno JA, et al. Prevalence and determinants of human papillomavirus infection in men attending vasectomy clinics in Mexico. *Int J Cancer* 2006;119:1934-9.
18. Hernandez BY, Wilkens LR, Zhu X, et al. Circumcision and human papillomavirus infection in men: a site-specific comparison. *J Infect Dis* 2008;197:787-94.
19. Nielson CM, Harris RB, Dunne EF, et al. Risk factors for anogenital human papillomavirus infection in men. *J Infect Dis* 2007;196:1137-45.
20. Baldwin SB, Wallace DR, Papenfuss MR, et al. Human papillomavirus infection in men attending a sexually transmitted disease clinic. *J Infect Dis* 2003;187:1064-70.
21. Nielson CM, Schiaffino MK, Dunne EF, Salemi JL and Giuliano AR. Associations between male anogenital human papillomavirus infection and circumcision by anatomic site sampled and lifetime number of female sex partners. *J Infect Dis* 2009;199:7-13.
22. Simen-Kapeu A, Kataja V, Yliskoski M, et al. Smoking impairs human papillomavirus (HPV) type 16 and 18 capsids antibody response following natural HPV infection. *Scand J Infect Dis* 2008;40:745-51.
23. Castellsague X, Bosch FX, Munoz N, et al. Male circumcision, penile human papillomavirus infection, and cervical cancer in female partners. *N Engl J Med* 2002;346:1105-12.
24. Burchell AN, Winer RL, de Sanjose S and Franco EL. Chapter 6: Epidemiology and transmission dynamics of genital HPV infection. *Vaccine* 2006;24 Suppl 3:52-61.
25. Monk BJ, Tewari KS. The spectrum and clinical sequelae of human papillomavirus infection. *Gynecol Oncol* 2007;107:S6-13.
26. Insinga RP, Dasbach EJ and Myers ER. The health and economic burden of genital warts in a set of private health plans in the United States. *Clin Infect Dis* 2003;36:1397-403.
27. Dinh TH, Sternberg M, Dunne EF and Markowitz LE. Genital warts among 18- to 59-year-olds in the United States, national health and nutrition examination survey, 1999--2004. *Sex Transm Dis* 2008;35:357-60.

28. Jeynes C, Chung MC and Challenor R. 'Shame on you' - the psychosocial impact of genital warts. *Int J STD AIDS* 2009;20:557-60.
29. Lacey CJ, Lowndes CM and Shah KV. Chapter 4: Burden and management of non-cancerous HPV-related conditions: HPV-6/11 disease. *Vaccine* 2006;24 Suppl 3:S3/35-41.
30. Wiley DJ, Douglas J, Beutner K, et al. External genital warts: diagnosis, treatment, and prevention. *Clin Infect Dis* 2002;35:S210-24.
31. Insinga RP, Dasbach EJ and Elbasha EH. Assessing the annual economic burden of preventing and treating anogenital human papillomavirus-related disease in the US: analytic framework and review of the literature. *Pharmacoeconomics* 2005;23:1107-22.
32. Lacey CJ. Therapy for genital human papillomavirus-related disease. *J Clin Virol* 2005;32 Suppl 1:S82-90.
33. Chan PK, Luk AC, Luk TN, et al. Distribution of human papillomavirus types in anogenital warts of men. *J Clin Virol* 2009;44:111-4.
34. Rubin MA, Kleter B, Zhou M, et al. Detection and typing of human papillomavirus DNA in penile carcinoma: evidence for multiple independent pathways of penile carcinogenesis. *Am J Pathol* 2001;159:1211-8.
35. Garland SM, Steben M, Sings HL, et al. Natural history of genital warts: analysis of the placebo arm of 2 randomized phase III trials of a quadrivalent human papillomavirus (types 6, 11, 16, and 18) vaccine. *J Infect Dis* 2009;199:805-14.
36. Aubin F, Pretet JL, Jacquard AC, et al. Human papillomavirus genotype distribution in external acuminata condylomata: a Large French National Study (EDiTH IV). *Clin Infect Dis* 2008;47:610-5.
37. Greer CE, Wheeler CM, Ladner MB, et al. Human papillomavirus (HPV) type distribution and serological response to HPV type 6 virus-like particles in patients with genital warts. *J Clin Microbiol* 1995;33:2058-63.
38. Brown DR, Schroeder JM, Bryan JT, Stoler MH and Fife KH. Detection of multiple human papillomavirus types in Condylomata acuminata lesions from otherwise healthy and immunosuppressed patients. *J Clin Microbiol* 1999;37:3316-22.

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