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Association of Known and Unknown Oncoviruses with External Genital Lesion (EGL) Manifestations in a Multinational Cohort of Men

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by

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DEDICATION

I dedicate this work to my parents, Abdul Rahman and Zareen Taj, who spent two decades of their life in hardship and day-to-day survival in refugee camps after the Soviets invaded Afghanistan. They never lost the hope of a bright future for their children. They taught me the importance of knowledge, hard-work, and the power of hope. Without their love and support I would be among the hundreds of thousands of children who lived in the same refugee camps and grew up without any basic literacy. Mom and dad I see heaven in your faces.

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ABSTRACT

Human papillomaviruses (HPV) are double-stranded, DNA, epitheliotropic viruses that infect skin and mucosal membranes. Over 200 types of HPV have been identified and classified into alpha (α), beta (β), gamma (γ), mu (μ), and nu (ν) genera. HPV in the genus α mainly infect mucosal membranes, cause the majority of the ano-genital cancers, and are widely studied. However, epidemiology of HPV in the other genera, which mainly infect skin, is poorly understood. Few studies have reported the seroprevalence of cutaneous HPV among healthy individuals, and to date, no study has prospectively examined the association between cutaneous HPV seropositivity and development of external genital lesions (EGLs) in men. The objectives of this study were to estimate the seroprevalence of cutaneous HPV types and investigate factors associated with the seropositivity, and evaluate the association between seropositivity to cutaneous HPV types and the risk of development of EGLs. Several studies have reported the seroprevalence of mucosal HPV types (6, 11, 16 and 18) in the 4-valent HPV vaccine among men. However, few studies have reported the seroprevalence of the five additional HPV types (31, 33, 45, 52 and 58) in the recently approved 9-valent HPV (9vHPV) vaccine specifically among men across a broad age range. Baseline data on seroprevalence prior to vaccine introduction and dissemination are needed to establish the effectiveness of vaccines over time. Also, this study estimated the seroprevalence of 9vHPV vaccine types and investigated factors associated with the seropositivity among men residing in Brazil, Mexico, and the United States (U.S.).

To estimate the seroprevalence of cutaneous HPV types and 9vHPV vaccine types, 600 men were randomly selected from the *HPV Infection in Men* (*HIM) Study*. To examine the association between seropositivity to cutaneous HPV types and development of EGLs, a case-control study of 163 incident EGL cases and 352 EGL-free controls nested in the *HIM* cohort was conducted. Cases were ascertained through visual inspection at each of up to 10 biannual clinical visits, confirmed through biopsy, and categorized into

condyloma, suggestive of condyloma, penile intraepithelial neoplasia (PeIN) and other EGLs. Archived serum specimens were tested for antibodies against 14 cutaneous HPV types, β types (5, 8, 12, 14, 17, 22, 23, 24, 38 and 47), α type 27, γ type 4, μ type1 and ν type 41, and 9vHP types (6, 11, 16, 18, 31, 33, 45, 52 and 58) using a glutathione S-transferase (GST) L1-based multiplex serology assay. Socio-demographic and sexual behavior data were collected through a questionnaire. Binomial proportions were used to estimate seroprevalence, and logistic regression was used to examine factors associated with seropositivity.

Overall, seroprevalence of ≥1 cutaneous HPV types was 65.4%, 1≥ β-HPV types was 39.0%, α-HPV 27 was 8.9%, γ-HPV 4 was 30.9%, μ-HPV 1 was 28.6%, and ν-HPV 41was 9.4%. Higher educational attainment was significantly associated with seropositivity to ≥1 cutaneous HPV types (adjusted odds ratio [AOR] 1.75 for ≥16 years of education vs. ≤12 years of education, 95% confidence interval [CI] 1.08-2.83), and seropositivity of ≥1 β-HPV types was significantly associated with increasing age (AOR 1.72 for men aged 31-44 years vs. men aged 18-30 years, 95% CI: 1.12–2.63,). Country of residence, circumcision status, and lifetime number of male anal sex partners were other factors significantly associated with various type-specific cutaneous HPV seropositivity. No statistically significant association was observed between grouped or individual cutaneous HPV seropositivity and the risk of development of EGLs across all pathological diagnoses. The seroprevalence of grouped and individual cutaneous HPV types was similar across different EGL categories and controls, with the most frequent types being ɤ-HPV 4, μ-HPV 1, and β-HPV 8. The seroprevalence of ≥1 9vHPV vaccine types was 28.3%, ≥1 high-risk types was 14.0%, five additional high-risk types was 11.2%, and low-risk types (6/11) was 17.4%. Compared to men with no male anal sex partners, men with ≥2 partners were two times more likely to be seropositive for grouped 9vHPV vaccine types, ≥1 high-risk types and ≥1 low-risk types, in addition to individual HPV types 6, 16, 33, and 58, with AORs ranging from 2.19 to 7.36. Older age, current smoking, and being single were other factors significantly positively associated with different grouped and type-specific seropositivity.

In conclusion, our data show that exposure to cutaneous HPV was common in men although different risk factors were independently associated with grouped and type-specific cutaneous HPV seropositivity. It appears that exposure to cutaneous HPV is not likely to increase the risk of EGLs among

men. Similarly, exposure to 9vHPV vaccine types was also common in men and seropositivity to 9vHPV vaccine types was positively associated with older age and lifetime number male anal sex partners.

CHAPTER ONE:

INTRODUCTION

Human papillomavirus (HPV) is one of the most common sexually transmitted infections worldwide.[1] In the U.S., the annual direct medical cost associated with the treatment and prevention of HPV related diseases is estimated at $5 billion.[2] Nearly all sexually active adults will get HPV at some point in their lives.[3,4] The prevalence of HPV among women differs markedly across different countries and populations. For example, one multinational study reported an age-adjusted prevalence of overall HPV infection of 1.5% in Spain and 25.6% in Nigeria.[5] The prevalence of HPV among men also varies from 1.3% to 72.9% in different areas of the world.[6] Although the human body clears most of the HPV infections without causing any clinical manifestations, a small proportion of HPV infections with certain types such as HPV 16 and 18 can persist and cause benign and malignant diseases such as genital warts and cancers.[4]

To date, more than 200 types of HPV have been identified and fully sequenced and the majority of these types are placed into three genera, alpha (α) predominantly found on mucosal surfaces, beta (β) and gamma (γ) which are found on skin and in hair follicles.[7-9] Over 40 types of HPV can infect cervix, linings of the vagina, vulva, penis, anus, rectum, mouth, throat, and other cutaneous epithelium.[10,11] Often HPV are classified into low-risk and high-risk based on their potential for causing cancer.[3,10] The International Agency for Research on Cancer (IARC) has categorized 13 types of HPV as carcinogens.[11] Nearly all cervical cancers are caused by the high-risk HPV, specifically types 16 and 18, and nearly all genital warts are caused by low-risk HPV, specifically HPV types 6 and 11.[4,11]

Emerging evidence shows that cutaneous HPV infection may increase the risk for squamous cell carcinoma (SCC) of the skin.[12-14] Each year about 3.5 million new cases of basal cell carcinoma (BCC) and SCC of the skin are diagnosed in the U.S.,[15] costing approximately half a billion in medical costs.[15-17]

Several studies have reported a positive association between anti cutaneous HPV serum antibodies and the risk of skin cancer.[12,18-20] Commonly reported types of HPV that infect skin cells are from beta (β), gamma (γ), mu (μ) and nu (ν) genera.[8] While the exact mechanism of the cutaneous HPV and SCC association is not clear, it is hypothesized that cutaneous HPV infection might modify the effect of ultra-violet (UV)-induced DNA damage, leading to apoptosis, accumulation of mutations, and eventually to SCC.[19]

Cutaneous HPV DNA has also been detected on the surfaces of penile intraepithelial neoplasia (PeIN),[21,22] penile cancer,[23,24] genital warts, other EGLs on UV-unexposed skin,[25] and on cutaneous warts.[26] Two hospital-based case-control studies reported the presence of cutaneous HPV types in nearly all PeIN cases.[21,22] PeIN are precursors of penile cancer.[27] Less prevalent in the U.S.,[28] penile carcinoma constitutes up to 10% of all cancers among men in the developing world,[29] and its incidence is on the rise in some European countries.[30-32] Although benign, genital warts (condyloma acuminata) are highly contagious and cause considerable amount of psychological discomfort and treatment related burden to patients. Both genital warts and PeIN (low and high-grade) are associated with an increased risk for carcinoma of the penis.[33,34]

The emerging role of cutaneous HPV in the etiology of SCC of the skin and the detection of β-cutaneous HPV in EGLs, strongly suggest a role for cutaneous HPV in the carcinogenesis of squamous epithelium both in the UV exposed and unexposed areas of the body. However, little data exist on the epidemiology and serology of cutaneous HPV, and their etiologic role in the development of SCC of the skin, EGLs, and penile cancer. HPV related lesions on male genitalia create reservoirs of infection and facilitate HPV transmission from men to women. Therefore, understanding the epidemiology of HPV infection among men is not only important for prevention of HPV related diseases in men, but also equally important in reducing the transmission of HPV infection from men to women.

In the U.S., the bivalent and 4-valent HPV vaccines are recommended for routine vaccination for girls at age 11 or 12 years, females age 13 through 26 years, and males age 13 through 21 years if they have not received the vaccine before.[35] Recently, the Advisory Committee on Immunization Practices (ACIP) added 9-valent HPV (9vHPV) vaccine to its recommendations.[36] Information on the epidemiology and

serology of HPV infections among men is needed for vaccine recommendations in other countries, monitoring the effectiveness of vaccines over time, and strategic planning for immunizations of high-risk groups. Although a number of studies have reported the seroprevalence of HPV types (6, 11, 16 and 18) among men in different countries,[37-42] few studies have examined other HPV types (i.e. 31, 33, 45, 52 and 58) in 9vHPV vaccine among men across the entire adult lifespan and old age.

SPECIFIC AIMS

The goal of this research was to estimate exposure to a wide array of cutaneous HPV and 9vHPV vaccine types in a large multinational cohort of men from three countries (Brazil, Mexico, and the U.S.) and to explore the etiologic role of cutaneous HPV types independently and jointly with mucosal HPV in the development of EGLs. The specific aims of this research were:

1) To estimate the seroprevalence of cutaneous HPV types in a sub-cohort of 600 men selected randomly from *the HPV infection in men (HIM) Study* participants at baseline and to investigate factors associated with the seropositivity.
2) To examine the association between seropositivity to cutaneous HPV types and the risk for subsequent development of EGLs and assess for interaction by serostatus to 9vHPV vaccine types in a case- control study nested in the *HIM* cohort.
3) To estimate the seroprevalence of 9vHPV vaccine types in a sub-cohort of 600 men selected randomly from *the HIM Study* participants at baseline and investigate factors associated with the seropositivity.

CHAPTER TWO:

MANUSCRIPT 1: SEROPREVALENCE OF CUTANEOUS HUMAN PAPILLOMAVIRUSES (HPV) AMONG MEN IN THE MULTINATIONAL HIM STUDY

ABSTRACT

Little is known about the seroprevalence of cutaneous HPV infection and associated factors among men. Data on cutaneous HPV seroprevalence is primarily derived from skin cancer case-controls studies. Few studies have reported the seroprevalence of cutaneous HPV among healthy individuals. This study investigated the seroprevalence of cutaneous HPV types and associated factors among men residing in Brazil, Mexico, and the U.S. Six hundred men were randomly selected from the *HPV Infection in Men (HIM) Study*. Archived serum specimens collected at enrollment were tested for antibodies against 14 cutaneous HPV types, β-HPV types (5, 8, 12, 14, 17, 22, 23, 24, 38 and 48), α-HPV 27, γ-HPV 4, μ-HPV1, ν-HPV 41 using a GST L1-based multiplex serology assay. Socio-demographic and sexual behavior data were collected by a questionnaire. Binomial proportions were used to estimate seroprevalence, and logistic regression was used to examine factors associated with seropositivity.

Overall, 65.4% of men were seropositive to ≥1 of the 14 cutaneous HPV types, and 39.0% were seropositive for ≥1 β-HPV types. Seroprevalence was 8.9%, 30.9%, 28.6%, and 9.4% for α-HPV 27, γ-HPV 4, μ-HPV 1, and ν-HPV 41, respectively. In multivariate analyses, seropositivity of any cutaneous HPV type was associated with higher education (adjusted odds ratio [AOR] 1.75; 95% CI: 1.08–2.83), and seropositivity of any β-HPV type was significantly associated with increasing age (AOR 1.72; 95% CI: 1.12– 2.63, for men aged 31-44 years vs. aged 18-30 years). Other factors associated with various type-specific cutaneous HPV seropositivity included country, circumcision, and lifetime number of male sexual partners. In conclusion, these data indicate that exposure to cutaneous HPV is common. Different risk

factors were independently associated with grouped and type-specific seropositivity. Future studies are needed to assess associations between seropositivity to cutaneous HPV types and the development of genital lesions among men.

INTRODUCTION

Human papillomavirus (HPV) is a non-enveloped double-stranded DNA virus that infects epithelial cells of the skin and mucus membranes.[43] HPV is one of the most common sexually transmitted infections (STIs) worldwide, and nearly all sexually active men and women acquire HPV at some point in their lives.[44] HPV infection can cause a wide range of benign and malignant diseases, such as anogenital warts, recurrent respiratory papillomatosis, and cancers of the cervix, anal canal, penis, and a subset of head and neck cancers.[45,46] To date, more than 200 types of HPV have been recognized and fully sequenced, and the majority of these types fall within three genera: alpha (α), predominantly found on mucosal surfaces, and beta (β) and gamma (γ), which are commonly found on skin and in hair follicles.[7,8]

Mucosal HPV types have been studied extensively with respect to their etiologic role in the development of genital warts[45,47] and several cancers.[14,46] However, emerging evidence suggests that cutaneous HPV infection may increase the risk for squamous cell carcinoma (SCC) of the skin.[19,20,48-50] Several studies have reported associations between cutaneous HPV serum antibodies and higher risk for SCC.[18-20,51] Commonly reported HPV types that infect cutaneous skin cells are from the, β, γ, μ, and ν genera.[8] While the exact mechanism of the cutaneous HPV and SCC association is unclear, it is hypothesized that cutaneous HPV infection might modify the effect of UV-induced DNA damage and apoptosis, leading to accumulation of mutations and SCC.[19] Furthermore, cutaneous HPV types have also been detected on the surface of external genital lesions[25] and penile cancer precursors in non-UV-exposed regions of the genital skin. Two hospital-based case-control studies have reported the presence of cutaneous HPV types in nearly all penile intraepithelial neoplasia (PeIN) cases.[21,22]

Despite its potential role in the development of SCC and PeIN, very little is known about the seroprevalence, associated factors, and natural history of cutaneous HPV infection among men. Previously,

we reported cutaneous HPV DNA prevalence detected on eyebrow hairs and skin swabs among U.S. men from the *HIM Study*.[52] The objective of the current study was to estimate the seroprevalence of cutaneous HPV types, including β (types 5, 8, 12, 14, 17, 22, 23, 24, 38 and 47), α (type 27), γ (type 4), μ (type 1), and ν (type 41) in a sub-cohort of healthy men from the *HIM Study* and to investigate factors associated with cutaneous HPV seropositivity.

METHODS

Study Population

This study included a sub-cohort of 600 men randomly selected from the *HIM Study*, a prospective cohort of the natural history of HPV infections in men. Simple random sampling method was used to select the sub-cohort because performing serology testing for the entire parent cohort was not viable and cost-effective. The *HIM Study* population and methods have been described previously[53,54] and a detailed description of the study population and recruitment strategies is also given in Appendix A1.7. Briefly, between July 2005 and September 2009, the *HIM Study* enrolled over 4000 men in the U.S., Brazil, and Mexico. Participants were eligible if they were: male, aged 18-70 years at baseline, and reported no previous diagnosis of penile or anal cancer, no previous diagnoses of anogenital warts, no current history or treatment for sexually transmitted infections including HIV, and no current discharges from the penis or burning sensation during urination. Participants were followed every six months for a median of four years of follow-up. At each study visit, participants completed a computer-assisted self-interviewed questionnaire (CASI), provided urine and blood samples, and underwent a clinical examination. A total of 3,695 HIM Study participants who provided a serum sample and completed the questionnaire at baseline were eligible for the current study. The study was approved by the Institutional Review Boards of the University of South Florida (Tampa, FL, U.S.), the Ludwig Institute for Cancer Research (Sao Paulo, Brazil), the Centro de Referencia e Treinamento em Doencas Sexualmente Transmissiveis e AIDS (Sao Paulo, Brazil), and the Instituto Nacional de Salud Publica de Mexico (Cuernavaca, Mexico).

Specimens and Data Collection

At the baseline visit, *HIM Study* participants provided detailed information on demographics (age, race, ethnicity, education, and marital status), socioeconomic status, medical history, smoking habits, alcohol consumption, and sexual history. Archived baseline serum samples from 600 participants were tested for seroreactivity to the L1 protein of 14 cutaneous HPV types, including β-types (5, 8, 12, 14, 17, 22, 23, 24, 38 and 47), α-type 27, γ-type 4, μ-type 1, and ν-type 41. Only 14 types were tested due to limited funds, and were carefully selected based on previous reports of their association with cutaneous SCC[19,48] and their detection on the surface of EGLs.[25] The antibody detection method was based on a glutathione S-transferase (GST) capture enzyme-linked immunosorbent assay (ELISA), in combination with fluorescent bead technology previously described.[55,56] To define type-specific HPV seropositivity, cut-off values (200 median fluorescence intensity [MFI] units) were applied, as previously described,[57,58] to allow for direct comparison with other studies using the same serologic assay.[57,58] Serology results for two subjects were inadequate and were therefore excluded from analysis, resulting in a final sample size of 598 men.

Statistical Analysis

Baseline participant characteristics, including demographic, lifestyle, and sexual behavior factors were compared between participants from Brazil, Mexico, and the U.S. using the Chi-square and Fisher exact tests for categorical variables (Table 2.1). Participants in the randomly selected sub-cohort (n=600) were also compared to the full *HIM* cohort (>4,000 men) on all baseline socio-demographic and sexual behavioral characteristics listed in Table 2.1. Seropositivity to any cutaneous HPV (any HPV) was defined as the proportion of men who were seropositive to at least one of the 14 types of HPV included in this study, and seropositivity to any β-HPV was defined as the proportion of men who were seropositive to at least one of the 10 β-HPV types. Type-specific and grouped seroprevalence was calculated for the overall study population and by country. Holms-Bonferroni correction of p-values was used to account for multiple comparisons.[59] Seropositivity to one, two, and three or more of the 14 cutaneous types was also estimated. To assess factors associated with seroprevalence of type-specific, any β-HPV, and any HPV in univariate

analyses, logistic regression was used to estimate odds ratios (ORs) and their 95% confidence intervals (CI). As seroprevalence was low (<10%) for some HPV types, type-specific logistic regression models were reported only for those types with seroprevalence of 10% or higher. To assess factors associated with cutaneous HPV seroprevalence, a number of factors listed in Table 1 were considered in modeling associations with any HPV and any β-HPV seroprevalence in multivariate models. A backward stepwise elimination logistic regression procedure was conducted, using significance at $p \leq 0.1$ for retention. Country and age were forced into the model due to study design. The final multivariable model included country, age, race, education, circumcision, number of lifetime female sex partners, and number of lifetime male sex partners. Using these adjustment factors, separate multivariable models were estimated for seroprevalence to any HPV, any β-HPV, and type-specific HPV. All analyses were performed using SAS 9.3.

RESULTS

Significant differences in socio-demographic and behavioral characteristics were observed by country for all listed variables (p-values < 0.05) except the number of male anal sex partners in the past six months among those reporting ever having had a male sex partner (Table 2.1). Participants in the randomly selected sub-cohort (n=600) were similar (p>0.05) to the full *HIM* cohort (>4,000 men) on all baseline socio-demographic and sexual behavioral characteristics (Table A2.1 in Appendices).

Overall, 65.4% participants were seropositive to one or more cutaneous HPV types (Table 2.1). Seroprevalence was 39.0% for at least one β-cutaneous HPV type, 8.9% for α-cutaneous type 27, 30.9% for γ-HPV type 4, 28.6% for μ-HPV type 1, and 9.4% for ν-HPV type 41 (Table 2.1, and Figures A2.1-2.4 in Appendices). The highest overall type-specific seroprevalence was observed for γ-HPV type 4 (30.9%), μ-HPV type 1 (28.6%) and β-HPV type 8 (20.4%). No significant difference (p-value=0.140) in the seroprevalence of any HPV was observed by country (Brazil [70.0%], U.S. [65.4%], and Mexico [60.7%]). Significant differences in cutaneous HPV seroprevalence by country were observed for any-β HPV, as well as for each HPV type-specific seroprevalence listed, with the exceptions of HPV 27 and HPV 41 (Table 2.1). Using the Holm-Bonferroni method, we observed significantly higher grouped β–HPV seroprevalence

among men in Brazil compared to Mexico (p-value<0.001). Table A2.2 in Appendices shows Wald's 95% confidence intervals for the seroprevalence estimates.

Figure 2.1 and 2.2 shows seropositivity to multiple HPV types. Averaged across all three countries, approximately 21.2% of men had antibodies to ≥3 HPV types, 14.4% to two HPV types, 29.8% to only one HPV type and 34.6% did not have antibodies against any type. Seropositivity to multiple HPV infections was statistically different by country (overall p-value<0.001) and remained significant after adjusting for multiple comparisons (p<0.05). Men in Brazil were more likely to be seropositive to ≥3 HPV types (34.0%) compared to men from the U.S. (22.3%) and Mexico (8.4%).

Factors associated with seroprevalence of cutaneous HPV types (any-HPV, any β-HPV, ϒ-HPV4, μ-HPV, β-HPV 8, β-HPV17, β-HPV 23, β-HPV 38, and β-HPV 47) are presented in Tables 2.3, Table 2.4, and for HPV types with <10% seroprevalence in Table A2.3 in Appendices. In the multivariate model, adjusting for all variables listed in Table 2.3, country was significantly associated with seropositivity of β-HPV 47 (AOR 2.67; 95% CI: 1.19 - 5.99), with men from Brazil more likely to be seropositive compared to men from the U.S. Compared to the youngest age group (18-30 years), mid-adult aged men (31-44 years) were significantly more likely to be seropositive for any-β HPV (AOR 1.72; 95% CI: 1.12 - 2.63), β-HPV 8 (AOR 2.28; 95% CI: 1.33 - 3.91), β-HPV 38 (AOR 2.01; 95% CI 1.09 - 3.72), and β-HPV 47 (AOR 2.00; 95% CI: 1.03 - 3.88), and men in the oldest age group (45-73 years) were more likely to be seropositive for β-HPV 8 and β-HPV 47. Higher educational attainment was significantly associated with any-HPV (AOR 1.75 for ≥16 years of education compared to ≤ 12 years of education, 95% CI: 1.08 - 2.83) and ϒ-HPV 4 (AOR 1.77 for ≥16 years of education compared to ≤ 12 years of education, 95% CI: 1.10 - 2.82). Circumcised men were more likely to be seropositive for ϒ-HPV 4 (AOR 1.72; 95%CI: 1.02 - 2.89) and β-HPV 47 (AOR 2.37; 95%CI: 1.21 - 4.67). Similarly, having ≥ 2 lifetime male sex partners was significantly independently associated with seropositivity of β-HPV 17 (AOR 2.22; 95% CI: 1.06 - 4.62) and β-HPV 23 (AOR 2.39; 95% CI: 1.07 - 5.33).

Figure 2.3 summarizes the results of multivariate analyses for any HPV, any β-HPV, and individual HPV types. Men from Brazil were consistently more likely than men from the U.S. to be seropositive to

cutaneous HPV types. Mid-adult and older aged men (31-44 and 45-73 years), men with ≥16 years of education, circumcised men, and men with ≥2 lifetime male sex partners were more likely to be seropositive to cutaneous HPV.

DISCUSSION

This is the first study to estimate cutaneous HPV seroprevalence in a healthy population of men from three countries. We reported seroprevalence of 14 cutaneous HPV types and factors associated with grouped and individual HPV seroprevalence in 600 men from *the HIM Study* residing in the U.S., Mexico and Brazil. More than 65% of men were seropositive for one or more cutaneous HPV infection types, and more than 39% to one or more beta HPV types. Elevated risk of cutaneous HPV seropositivity was observed among men who were mid-adult and older ages, from Brazil, attained ≥16 years of education, circumcised, and reported ≥ 2 lifetime male sex partners.

Previous to our population-based study, other estimates of cutaneous HPV seroprevalence were available only from control series of case-control studies of skin cancer. Due to differences in populations studied, age distribution, and the number of HPV types assessed, a direct comparison to the published literature is not possible. A cross-sectional study[60] of cutaneous HPV seroprevalence among men and women undergoing a routine skin cancer screening in Tampa, Florida reported an overall seroprevalence for any HPV of 96.0% and 76.0% for any β HPV in men, which is higher than what we observed at 65.4% and 39.0%, respectively. Differences between this and the cross-sectional study[60] may be due to older age of the study population in the cross-sectional study , recruited from a skin cancer screening clinic, and that analysis of the overall seroprevalence included 36 types of HPV compared to 14 types in our study. Type-specific seroprevalence estimates in the cross-sectional study[60] were also slightly higher for γ-HPV 4 (46.0% vs. 39.0% in our study), μ-HPV 1 (37.0% vs. 28.6% in our study), and ν-HPV 41 (14.0% vs. 9.4% in our study). The ubiquity of the cutaneous HPV suggests that cutaneous HPV might be a commensal component of the microbiological flora of the skin.[61,62] One case-control study of SCC[18] from Sweden and Austria reported seroprevalence estimates for controls that were comparable to those in our study for any

β-HPV (42.0%) and slightly higher seroprevalence estimates for type-specific HPV; however, the study population in that case-control study was older, and the number of β-HPV tested were 8 compared to 10 in our study. Some of the type-specific seroprevalence estimates in our study were also comparable with seroprevalence estimates reported from the control group of a systematic review[63] of 15 studies examining the associations between cutaneous HPV and skin cancer, β-HPV 8 (22.8% vs. 20.4% in our study), β-HPV 23 (12.4% vs. 12.2%), α-HPV 27 (12.5% vs. 8.9), γ-HPV 4 (33.7% vs. 30.9%), μ-HPV 1 (27.1% vs. 28.6%), and ν-HPV 41 (11.8% vs.9.4%) respectively.

The increase in seroprevalence with increasing age observed in our study is consistent with studies of cutaneous HPV DNA detection conducted in the general population.[64,65] Boxman and colleagues[64] found strong associations between increasing age and cutaneous HPV DNA prevalence in the general population, with 29% DNA prevalence of any cutaneous HPV among 25-39-year-olds, 42% in 40-59-year-olds, and 65% in 60-79-year-olds. A study by De-Koning et al.[65] demonstrated a marginally significant association between increasing age and DNA prevalence of grouped beta HPV in the general population in Italy and Australia. However, neither study assessed associations between age and individual HPV types. Furthermore, few skin cancer case-control studies have assessed factors associated with cutaneous HPV seroprevalence in controls. Stujik et al. and Hampras et al. reported a significant association between increasing age and cutaneous HPV DNA prevalence.[12,52] However, Iannacone and colleagues did not report an association between age and seropositivity to cutaneous HPV.[60] In multiple studies of mucosal HPV seroprevalence, seroprevalence has been shown to increase significantly with age.[39]

Cutaneous HPV seroprevalence in the current study also varied across geographical regions, with men in Brazil having the highest seroprevalence and men in Mexico the lowest for certain β HPV types. This is consistent with our previous findings where we reported higher seroprevalence among Brazilian men for mucosal HPV genotypes.[39] Other multi-center studies have also reported geographic variation of cutaneous HPV DNA prevalence.[7,65,66] It is possible that the observed geographical variation is reflective of the differences in individual immune response, environmental factors, sexual behaviors, and other unobserved factors. The observed difference in our study is unlikely due to specimen collection or antibody

testing techniques as study protocols were uniformly applied across the three study centers, and the serum specimens were tested using the same assay in a single laboratory.

A pattern of elevated risk of cutaneous HPV seroprevalence among circumcised men was observed, specifically for γ-HPV 4, and β types 5, 14, 24 and 47. To our knowledge this is the first study to compare seroprevalence of cutaneous HPV between circumcised and uncircumcised men. In a previous study of *the HIM study* population, Sichero et al. did not find cutaneous HPV DNA to be associated with circumcision.[7] Similarly, Albero et al. did not find an association between circumcision and DNA prevalence of any mucosal HPV, oncogenic HPV, and unclassified HPV types, but found a weak inverse association for non-oncogenic HPV types.[67] However, Lu et al. reported an association between seroprevalence of mucosal HPV type 11 and circumcision with lower seroprevalence among circumcised men.[39] These findings suggest that the role of circumcision and HPV prevalence is complex and may depend on HPV type (cutaneous or mucosal), anatomic site of infection, and whether prevalence is based on DNA detection or antibody response. Although the inverse association of circumcision with mucosal HPV prevalence has been reported in other populations,[68,69] the role of circumcision and cutaneous HPV prevalence is largely unknown. One possible explanation for this positive association could be the keratinization of the glans penis after circumcision. At birth, the glans is covered by the foreskin or prepuce, which is often removed from infants by circumcision.[70] The glans is non-keratinized before circumcision and becomes keratinized after circumcision.[70,71] In contrast to mucosal HPV, cutaneous HPV mainly infect skin and keratinized epithelium. Alternatively, it is also possible that circumcised penile tissues might have different immunogenic response to HPV than uncircumcised penile tissues. However, men in Brazil were mostly uncircumcised but had higher seroprevalence estimates. Cutaneous HPV DNA has been commonly detected on the normal skin,[65] skin tumors,[72] oral cavity,[73] and anogenital tract.[7] Given that the vast majority of studies have examined cutaneous HPV from non-genital anatomic sites, other modes of transmission besides skin to skin contact, might warrant further investigations.

Education was significantly associated with any HPV, γ-HPV type 4, and β-HPV type 24. Men with higher education were more likely to be seropositive, an association also observed in the study of

Farzan et al. for some types of cutaneous HPV[74] and in the study of Molano et al. for mucosal HPV types.[75] It is likely that education functions as a marker of lifestyle characteristics that may influence cutaneous HPV acquisition and natural history. It is possible that men with higher education have more opportunities for meeting new partners[76] and increased skin to skin contact, which could result in a higher level of exposure to HPV. Seroprevalence to beta types 17, 23 and 24 were positively associated with the number of lifetime male sexual partners. This finding is consistent with previous studies of mucosal HPV,[39,77,78] and could be explained that a higher number of sexual partners increases the probability of viral exposure, transmission, and risk of infection.

The major strengths of this study are its large sample size, estimation of prevalence based on antibodies against L1 HPV capsid protein in a single laboratory simultaneously by multiplex serology, and the availability of detailed demographic and sexual behavior data. We also acknowledge some limitations. Considering the cross-sectional nature of current study, causal associations could not be determined. Another limitation is that measurement of antibodies against L1 viral protein do not necessarily represent current infection with HPV. Furthermore, due to lack of information of the serodynamics of cutaneous HPV, the impact of seroconversion, clearance of infections, and waning of antibodies with time could not be assessed; however, these are limitations inherent to all seroprevalence studies. Despite its limitations, the current study provides important data and contributes to the understanding of the distribution and associated factors of cutaneous HPV, which is largely unknown.

Table 2.1. Participant characteristics by country of residence (the U.S. Brazil, Mexico)

	Overall (N=598)		Country						
			U.S. (N=184)		Brazil (N=200)		Mexico (N=214)		
Characteristic	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**p[a]**
Age, Years									
18-30	259	43.3	113	61.4	74	37.0	72	33.6	**<.001**
31-44	256	42.8	50	27.2	99	49.5	107	50.0	
45-70	83	13.9	21	11.4	27	13.5	35	16.4	
Race									
White	269	45.0	111	60.3	146	73.0	12	5.6	**<.001**
Black	78	13.0	40	21.7	38	19.0	0	0.0	
Asian	17	2.8	15	8.2	2	1.0	0	0.0	
American Indian/Alaska Native	9	1.5	0	0.0	9	4.5	0	0.0	
Other	218	36.5	17	9.2	4	2.0	197	92.1	
Not reported	7	1.2	1	0.5	1	0.5	5	2.3	
Ethnicity									
Hispanic	282	47.2	22	12.0	49	24.5	211	98.6	**<.001**
Non-Hispanic	305	51.0	162	88.0	143	71.5	0	0.0	
Not reported	11	1.8	0	0.0	8	4.0	3	1.4	
Education, Years									
≤12	287	48.0	44	23.9	112	56.0	131	61.2	**<.001**
13-15	159	26.6	94	51.1	45	22.5	20	9.3	
≥16	149	24.9	45	24.5	43	21.5	61	28.5	
Not reported	3	0.5	1	0.5	0	0.0	2	0.9	
Marital Status									
Single/never married	259	43.3	125	67.9	89	44.5	45	21.0	**<.001**
Married/cohabitating	278	46.5	37	20.1	84	42.0	157	73.4	
Divorced/separated/widowed	58	9.7	21	11.4	25	12.5	12	5.6	
Not reported	3	0.5	1	0.5	2	1.0	0	0.0	
Smoking Status									
Never	339	56.7	111	60.3	129	64.5	99	46.3	**0.003**
Former	113	18.9	30	16.3	32	16.0	51	23.8	
Current	146	24.4	43	23.4	39	19.5	64	29.9	
Alcohol, No. Drinks/Month									
0	141	23.6	29	15.8	55	27.5	57	26.6	**0.016**
1–30	279	46.7	82	44.6	89	44.5	108	50.5	
31-60	58	9.7	22	12.0	18	9.0	18	8.4	
≥61	110	18.4	48	26.1	34	17.0	28	13.1	
Not reported	10	1.7	3	1.6	4	2.0	3	1.4	
Circumcision									
No	389	65.1	32	17.4	165	82.5	192	89.7	**<.001**
Yes	209	34.9	152	82.6	35	17.5	22	10.3	
Sexual Orientation[b]									
MSW	524	87.6	167	90.8	154	77.0	203	94.9	**<.001**
MSM	51	8.5	6	3.3	37	18.5	8	3.7	
MSWM	15	2.5	4	2.2	9	4.5	2	0.9	
Missing	8	1.3	7	3.8	0	0.0	1	0.5	
No. of Female LTP[c]									
0	60	10.0	29	15.8	15	7.5	16	7.5	**<.001**
1-3	126	21.1	42	22.8	26	13.0	58	27.1	
4-18	246	41.1	63	34.2	74	37.0	109	50.9	
>=19	126	21.1	41	22.3	65	32.5	20	9.3	
Not reported	40	6.7	9	4.9	20	10.0	11	5.1	
No. of Male Anal LTP[c]									
0	507	84.8	176	95.7	135	67.5	196	91.6	**<.001**
1	28	4.7	1	0.5	17	8.5	10	4.7	
2 or more	58	9.7	7	3.8	43	21.5	8	3.7	
Not reported	5	0.8	0	0.0	5	2.5	0	0.0	
No. of female sex partners in the past 6 months[d]									
0	115	21.4	26	16.8	34	18.4	55	27.8	**<.001**
1	241	44.8	80	51.6	74	40.0	87	43.9	
≥2	162	30.1	49	31.6	72	38.9	41	20.7	
Not reported	20	3.7	0	0.0	5	7.6	15	7.6	

(Continued on next page)

Table 2.1. (Continued)

Characteristic	Overall (598)		Country: U.S. (N=184)		Country: Brazil (N=200)		Country: Mexico (N=214)		
	n	%	n	%	n	%	n	%	p[a]
No. of male sex partners in the past 6 months[d]									
0	55	60.4	2	25.0	39	60.0	14	77.8	0.133
1	13	14.3	2	25.0	9	13.9	2	11.1	
≥2	21	23.1	4	50.0	16	24.6	1	5.6	
Not reported	2	2.2	0	0.0	1	1.5	1	5.6	

a. Chi-square and Fisher's exact tests were used to calculate p values.
b. MSW=men having sex with women, MSM=men having sex with men, MSWM=men having sex with women and men.
c. LTP = Life time partners
d. Among those reporting ever having a female sex partner
Note: Significant p-value highlighted in bold

Table 2.2. Type-specific cutaneous HPV seroprevalence among men residing in the U.S., Brazil, and Mexico[a,b]

Type of HPV	Overall (N=598) Seroprevalence %	U.S. (n=184) Seroprevalence %	Brazil (n=200) Seroprevalence %	Mexico (n=214) Seroprevalence %
Any HPV	65.4	65.8	70.0	60.7
Any β	39.0	39.1	48.5	29.9
β-HPV 5	7.7	6.5	15.0	1.9
β-HPV 8	20.4	21.2	29.5	11.2
β-HPV 12	6.0	3.3	13.0	1.9
β-HPV 14	5.7	3.8	11.0	2.3
β-HPV 17	13.0	10.9	19.5	8.9
β-HPV 22	6.9	6.5	11.0	3.3
β-HPV 23	12.2	12.0	19.5	5.6
β-HPV 24	7.7	7.1	12.5	3.7
β-HPV 38	13.9	14.1	21.0	7.0
β-HPV 47	12.4	13.0	16.5	7.9
α-HPV 27	8.9	9.2	11.5	6.1
γ-HPV 4	30.9	33.2	37.5	22.9
μ-HPV 1	28.6	37.5	34.5	15.4
ν-HPV 41	9.4	7.6	12.0	8.4

a. 600 subjects were randomly selected at baseline from *the HIM Study* to participate in the cutaneous HPV seroprevalence study. Seroprevalence data for 2 subjects were not available; therefore, they were excluded from analysis.
b. Chi-square and fisher exact tests were used to calculate p-values; Except Any-HPV, α-HPV 27, and ν-HPV 41, all other HPV types differ by country (p-value <0.05)

Table 2.3. Factors independently associated with any cutaneous HPV, β, γ, and μ HPV seroprevalence among men residing in the U.S., Brazil and Mexico[a]

	Any-HPV[b]		Any β-HPV[b]		γ-HPV 4		μ-HPV 1	
Characteristics	**OR**	**AOR (95%CI)**	**OR**	**AOR (95%CI)**	**OR**	**AOR (95%CI)**	**OR**	**AOR (95%CI)**
Country								
U.S.	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Brazil	1.21	1.41 (0.76 - 2.63)	1.46	1.44 (0.80 - 2.61)	1.21	1.76 (0.95 - 3.26)	0.88	1.17 (0.63 - 2.17)
Mexico	0.81	0.75 (0.31 - 1.85)	0.66	0.40 (0.16 - 0.99)	**0.60**	0.81 (0.31 - 2.17)	**0.30**	0.47 (0.17 - 1.29)
Age, Years								
18-30	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
31-44	1.07	1.20 (0.79 - 1.83)	**1.54**	**1.72 (1.12 - 2.63)**	1.04	1.09 (0.70 - 1.71)	0.87	1.12 (0.70 - 1.79)
45-70	1.08	1.04 (0.58 - 1.85)	1.54	1.63 (0.91 - 2.92)	1.51	1.39 (0.77 - 2.53)	1.02	1.33 (0.71 - 2.49)
Race								
White	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Black	0.63	0.56 (0.32 - 0.98)	0.80	0.72 (0.41 - 1.28)	0.59	0.58 (0.31 - 1.06)	0.69	0.64 (0.35 - 1.17)
Asian/American Indian/Alaska Native	1.46	1.70 (0.59 - 4.88)	1.28	1.47 (0.60 - 3.59)	0.85	1.06 (0.42 - 2.68)	**2.38**	**2.78 (1.12 - 6.89)**
Other	0.70	1.01 (0.44 - 2.30)	0.58	1.53 (0.65 - 3.60)	**0.50**	0.86 (0.35 - 2.14)	**0.35**	0.70 (0.27 - 1.80)
Education								
≤12	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
13-15	1.08	0.89 (0.55 - 1.44)	1.26	1.19 (0.74 - 1.93)	1.22	1.06 (0.64 - 1.76)	**1.63**	0.91 (0.54 - 1.53)
≥16	**1.75**	**1.75 (1.08 - 2.83)**	**1.53**	1.46 (0.93 - 2.30)	**1.91**	**1.77 (1.10 - 2.82)**	1.50	1.41 (0.86 - 2.32)
Circumcision								
No	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Yes	1.15	1.36 (0.80 - 2.30)	1.12	1.23 (0.74 - 2.04)	**1.57**	**1.72 (1.02 - 2.89)**	**1.96**	1.50 (0.88 - 2.56)
No. of Female Sex Lifetime Partners								
0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
1-3	0.69	0.69 (0.34 - 1.40)	0.73	0.90 (0.46 - 1.79)	0.98	0.94 (0.45 - 1.98)	1.10	1.24 (0.59 - 2.62)
4-18	0.79	0.79 (0.41 - 1.55)	0.93	1.03 (0.54 - 1.94)	1.30	1.26 (0.63 - 2.49)	1.12	1.25 (0.62 - 2.52)
≥19	0.69	0.56 (0.27 - 1.15)	0.98	0.77 (0.38 - 1.54)	1.48	1.03 (0.49 - 2.18)	1.10	0.85 (0.39 - 1.84)
No. of Male Sex Lifetime Partners								
0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
1	0.86	0.86 (0.36 - 2.09)	1.77	1.91 (0.80 - 4.56)	0.76	0.69 (0.26 - 1.84)	0.69	0.69 (0.24 - 1.97)
≥2	1.74	1.27 (0.63 - 2.54)	**2.34**	1.81 (0.97 - 3.38)	1.40	1.05 (0.55 - 2.02)	1.23	0.85 (0.43 - 1.68)

a. Final model was selected through backward stepwise elimination with significance level of p≤0.1 for retention in the model. Country, age, race, marital status, education, smoking alcohol consumption, circumcision, # of female LTP, and # of male LTP were loaded to the model while forcing country and age (design factors) to stay in the model

b. 'Any-HPV' category included men seropositive for at least one HPV types, and 'Any β HPV' category included men seropositive for at least one β HPV types.

Note: OR= unadjusted OR; AOR = adjusted odds ratio; CI = confidence interval; LTP = lifetime sex partners; significant results are marked in bold

Table 2.4. Factors independently associated with any cutaneous HPV, β, γ, and μ HPV seroprevalence among men residing in the U.S., Brazil and Mexico[a]

	β-HPV 8		β-HPV 17		β-HPV 23		β-HPV 38		β-HPV 47	
Characteristics	**OR**	**AOR (95%CI)**	**OR**	**AOR (95%CI)**	**OR**	**AOR (95%CI)**	**OR**	**AOR (95%CI)**	**OR**	**AOR (95%CI)**
Country										
U.S.	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Brazil	1.56	1.24 (0.62 - 2.48)	**1.99**	1.98 (0.87 - 4.55)	**1.78**	1.37 (0.58 - 3.26)	1.62	1.10 (0.49 - 2.44)	1.32	**2.67 (1.19 - 5.99)**
Mexico	**0.47**	0.52 (0.16 - 1.71)	0.80	0.81 (0.19 - 3.53)	**0.44**	0.28 (0.06 - 1.26)	**0.46**	0.20 (0.06 - 0.75)	0.58	0.42 (0.11 - 1.64)
Age, Years										
18-30	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
31-44	**2.13**	**2.28 (1.33 - 3.91)**	1.68	1.86 (0.98 - 3.51)	**1.81**	1.79 (0.90 - 3.53)	**1.86**	**2.01 (1.09 - 3.72)**	1.34	**2.00 (1.03 - 3.88)**
45-73	**2.45**	**2.83 (1.41 - 5.65)**	1.90	1.95 (0.85 - 4.48)	1.19	1.23 (0.47 - 3.26)	1.66	1.77 (0.77 - 4.05)	**2.41**	**3.16 (1.41 - 7.06)**
Race										
White	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Black	0.73	0.64 (0.33 - 1.25)	0.91	0.83 (0.38 - 1.83)	0.65	0.68 (0.28 - 1.66)	0.78	0.80 (0.37 - 1.73)	0.84	0.85 (0.38 - 1.89)
Asian/American Indian/Alaska Native	0.79	1.02 (0.35 - 3.01)	1.32	1.40 (0.43 - 4.56)	**3.58**	**4.75 (1.71 - 13.14)**	1.42	2.23 (0.77 - 6.44)	1.04	1.04 (0.28 - 3.83)
Other	**0.33**	0.57 (0.19 - 1.71)	**0.53**	0.93 (0.24 - 3.65)	**0.36**	1.25 (0.31 - 5.12)	**0.43**	1.48 (0.44 - 5.02)	**0.55**	2.29 (0.63 - 8.25)
Education										
≤12	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
13-15	0.90	0.82 (0.45 - 1.51)	1.10	1.32 (0.66 - 2.62)	1.53	1.25 (0.58 - 2.69)	1.07	0.99 (0.50 - 1.96)	1.43	1.63 (0.82 - 3.26)
≥16	1.50	1.28 (0.74 - 2.21)	1.25	1.17 (0.61 - 2.28)	**2.02**	2.02 (1.00 - 4.07)	**1.80**	1.56 (0.83 - 2.92)	1.54	1.36 (0.69 - 2.66)
Circumcision										
No	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Yes	1.21	1.09 (0.60 - 1.98)	1.05	1.38 (0.68 - 2.77)	1.03	1.24 (0.59 - 2.59)	0.94	0.81 (0.40 - 1.63)	**1.70**	**2.37 (1.21 - 4.67)**
No. of Female LTP										
0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
1-3	0.55	0.69 (0.30 - 1.60)	0.78	1.10 (0.43 - 2.80)	0.80	1.13 (0.37 - 3.47)	1.02	1.74 (0.60 - 5.02)	0.88	1.02 (0.38 - 2.73)
4-18	0.88	0.90 (0.42 - 1.91)	0.69	0.83 (0.34 - 2.01)	1.05	1.53 (0.55 - 4.23)	1.34	2.02 (0.75 - 5.40)	1.01	0.94 (0.38 - 2.31)
≥19	1.07	0.63 (0.28 - 1.45)	0.78	0.63 (0.24 - 1.67)	1.26	1.10 (0.37 - 3.33)	1.43	1.23 (0.43 - 3.56)	0.95	0.58 (0.21 - 1.59)
No. of Male LTP										
0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
1	0.98	0.86 (0.29 - 2.52)	0.95	0.61 (0.13 - 2.77)	2.39	2.43 (0.80 - 7.45)	1.24	1.14 (0.35 - 3.68)	0.56	0.58 (0.13 - 2.69)
≥2	**2.96**	1.80 (0.92 - 3.51)	**3.27**	**2.22 (1.06 - 4.62)**	**2.78**	**2.39 (1.07 - 5.33)**	**2.84**	1.97 (0.94 - 4.16)	1.52	0.95 (0.41 - 2.24)

a. Final model was selected through backward stepwise elimination with significance level of p≤0.1 for retention in the model. Country, age, race, marital status, education, smoking alcohol consumption, circumcision, # of female LTP, and # of male LTP were loaded to the model while forcing country and age (design factors) to stay in the model

b. 'Any-HPV' category included men seropositive for at least one HPV genotype, and 'Any β HPV' category included men seropositive for at least one β HPV types.

Note: OR= unadjusted OR; AOR = adjusted odds ratio; CI = confidence interval; LTP = lifetime sex partners; significant results are marked in bold

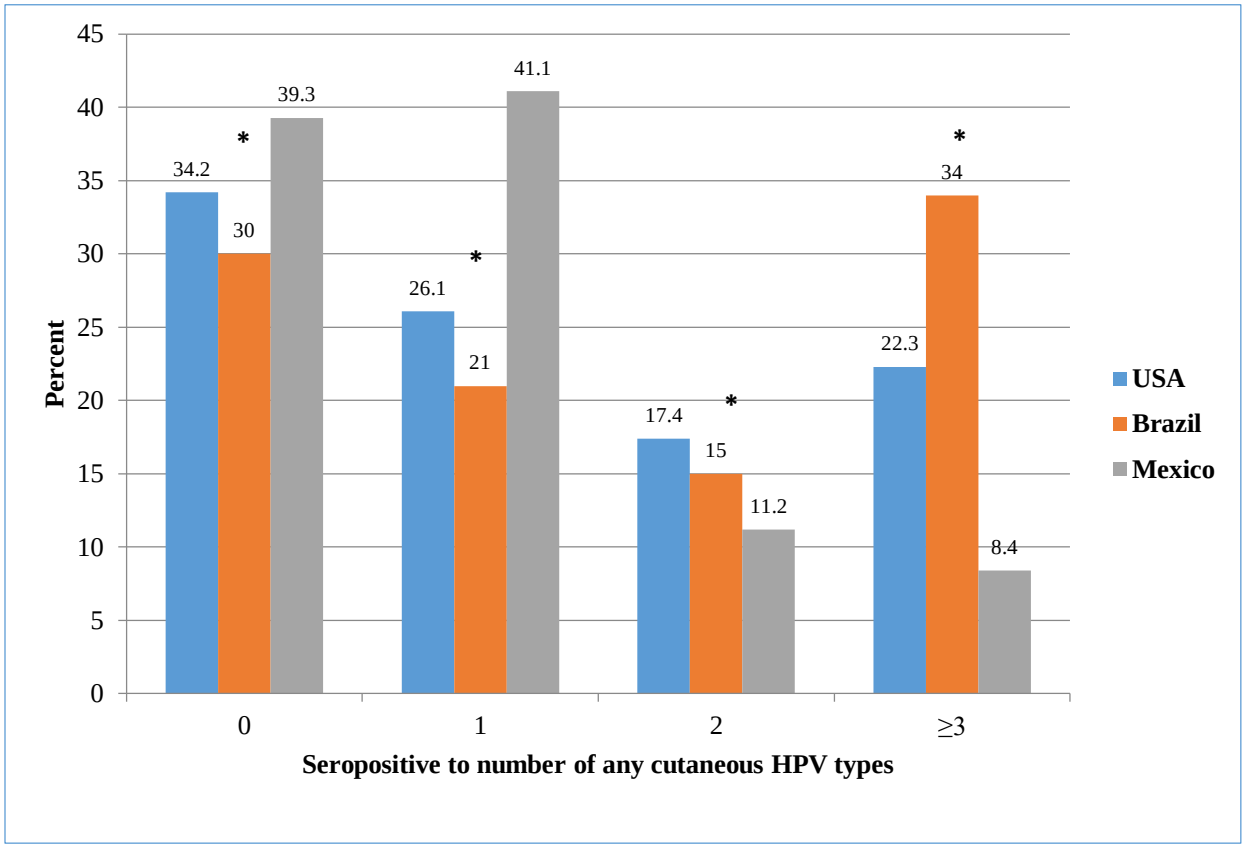

Figure 2.1. Seropositivity for 0, 1, 2, and ≥3 cutaneous HPV types by country

If a person was seronegative for all 14 HPV types, he was categorized in the seropositivity to '0 HPV' group. If a person was seropositive for only 1 HPV type out of the 14 types, he was categorized in the seropositivity to '1 HPV' group. If a person was seropositive for 2 HPV types, he was categorized in the '2 HPV' group, and if a person was seropositive for 3 or more types, he was categorized in the '≥ 3' group. Chi-square p-value represents differences in each category by country. Distributions of seropositivity to 0, 1, 2, ≥3 HPV types significantly differed by country in all categories (Overall p-value <0.05). * Highlights significant p-values.

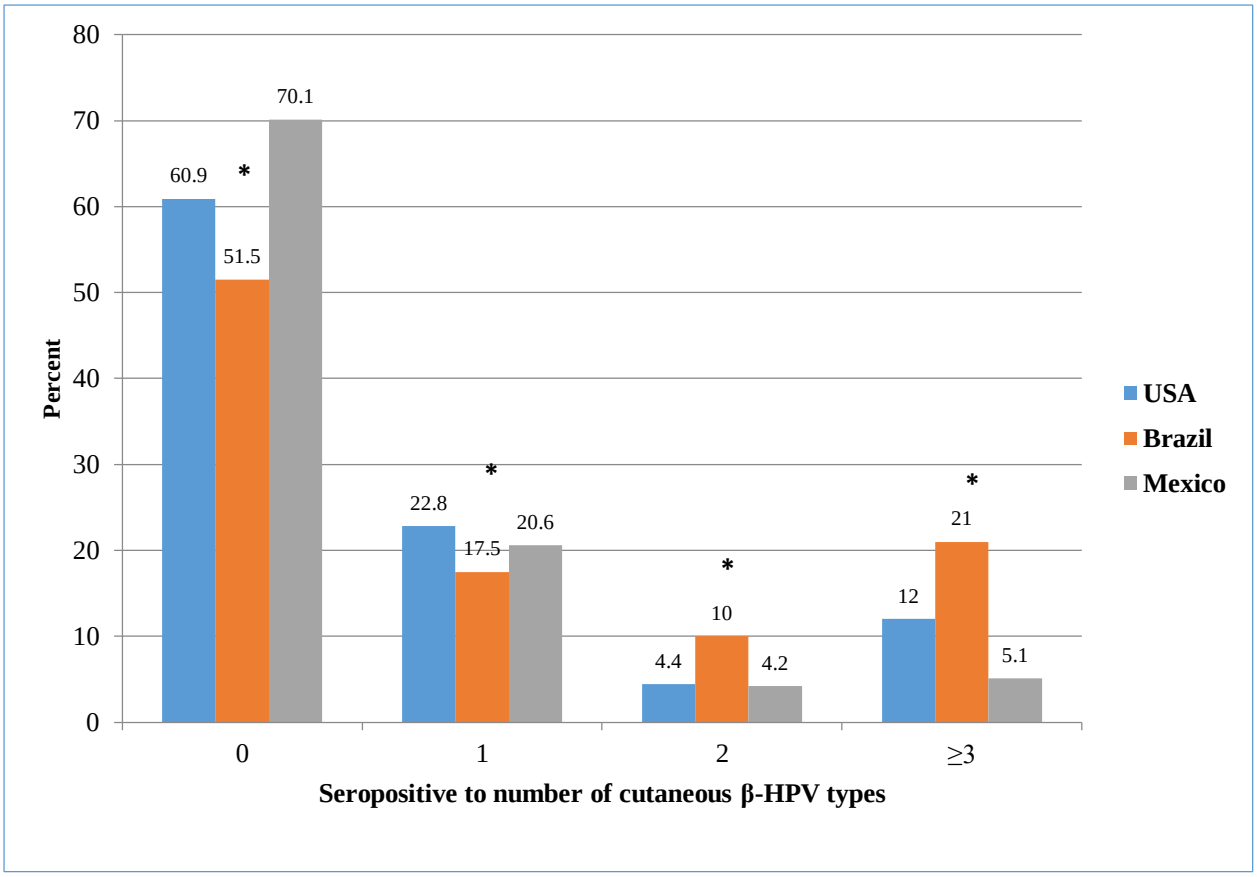

Figure 2.2. Seropositivity for 0, 1, 2, and ≥3 β HPV types by country

If a person was seronegative for all 10 **β** types, he was categorized in the seropositivity to '0 HPV' group. If a person was seropositive for only 1 type out of the 10 **β** types, he was categorized in the seropositivity to '1 HPV' group. If a person was seropositive for 2 **β** types, he was categorized in the '2 HPV' group, and if a person was seropositive for 3 or more **β** types, he was categorized in the '≥ 3' group. Chi-square p-value represents differences in each category by country. Distributions of seropositivity to 0, 1, 2, ≥3 **β** HPV types significantly differed by country in all categories (Overall p-value <0.05). * Highlights significant p-values.

Characteristics	Any-HPV[a]	Any β HPV[b]	α-HPV 27	γ-HPV 4	μ-HPV 1	ν-HPV 41	β-HPV 5	β-HPV 8	β-HPV 12	β-HPV 14	β-HPV 17	β-HPV 22	β-HPV 23	β-HPV 24	β-HPV 38	β-HPV 47
Country																
U.S.																
Brazil																
Mexico																
Age, Years																
18-30																
31-44																
45-73																
Race																
White																
Black																
Asian/AI/AN																
Other																
Education																
≤12																
13-15																
≥16																
Circumcision																
No																
Yes																
No. of Female LTP[c]																
0																
1-3																
4-18																
≥19																
No. of Male LTP[c]																
0																
1																
≥2																

Figure 2.3. Factors independently associated with cutaneous HPV seroprevalence among men residing in the U.S., Brazil, and Mexico –graphical representation of the multivariate models

Blue color represents the reference categories, white color represents not estimated, green color represents adjusted odds ratio (AOR) and 95% confidence interval (CI) with protective effect for non-significant associations, dark red color represents AOR and 95% CI with increased risk for significant associations, and light red color represents AOR and 95% CI with increased risk for non-significant associations. a. Any-HPV = seropositivity to ≥1 HPV types; b. Any-β HPV =seropositivity to ≥1 β HPV types; c. LTP = lifetime sex partners

CHAPTER THREE:

MANUSCRIPT 2: SEROPREVALENCE OF CUTANEOUS HUMAN PAPILLOMAVIRUSES (HPV) AND THE RISK OF EXTERNAL GENITAL LESIONS (EGLS) IN MEN - A NESTED CASE CONTROL STUDY

ABSTRACT

A variety of cutaneous human papillomaviruses (HPV) are detectable in genital epithelial lesions in men and non-melanoma skin cancer patients. It remains unclear whether these viruses are associated causally with disease. To date, no study has prospectively examined the association between cutaneous HPV seropositivity and development of external genital lesions (EGLs) in men. The objective of this study mine the association between seropositivity to cutaneous HPV types and the risk of subsequent development of EGLs. A nested case-control study including 163 incident EGL cases and 352 EGL-free controls in *the HPV Infection in Men (HIM) Study* cohort was conducted. Cases were ascertained at each of up to 10 biannual clinical visits and verified through biopsy and pathological diagnoses. EGLs were categorized as condyloma, suggestive of condyloma, penile intraepithelial neoplasia (PeIN), and other EGLs. Archived serum specimens collected at baseline were tested for antibodies against 14 cutaneous HPV types (β types [5, 8, 12, 14, 17, 22, 23, 24, 38, and 47], α type 27, γ type 4, μ type 1, and ν type 41) using a GST L1-based multiplex serology assay. Socio-demographic and sexual behavior data were collected through a questionnaire. Using logistic regression, adjusted odds ratios (AOR) and 95% confidence intervals (CI) were estimated.

Overall, seropositivity to ≥ 1 cutaneous HPV type (any-HPV) and ≥1 β types (any-β) was 58.3% and 37.5% among other EGL cases, 71.6% and 46.8% among condyloma, 66.8% and 50.0% among PeIN, and 71.9% and 38.4% among controls, respectively. Type-specific seropositivity was most common for ϒ-HPV 4, μ-HPV 1, and β-HPV 8. No statistically significant association was observed between any-HPV, any-β, and type-specific HPV seropositivity and subsequent development of EGLs across all pathological

diagnoses. In conclusion, overall, seropositivity to cutaneous HPV was common among men; however, it appears that cutaneous HPV is not associated with the development of genital lesions in men.

INTRODUCTION

Over 200 types of human papillomaviruses (HPV) have been identified,[79] and classified into five genera: alpha (α), beta (β), gamma (γ) mu (μ) and nu (ν).[8,80] The majority of HPV in the genus α infect mucosal membranes but some types are also detected in cutaneous skin.[18,81] HPV with cutaneous tropism are categorized into genus β, including some types initially referred to as epidermodysplasia verruciformis (EV) types, which have been associated with skin lesions, particularly in immune-suppressed individuals. Genus γ infects cutaneous skin and creates intra-cytoplasmic inclusion bodies. Finally, genera μ and ν are also associated with skin lesions.[18,81]

Emerging evidence shows that cutaneous HPV infection may increase the risk of squamous cell carcinoma (SCC) of the skin.[49,82] Several studies have reported a positive association between cutaneous HPV seropositivity and DNA detection and the risk of SCC.[18-20,48,83] It is hypothesized that cutaneous HPV infection might modify the effect of ultraviolet (UV) radiation induced DNA damage and apoptosis leading to accumulation of mutations and SCC.[84] Cutaneous HPV DNA has also been detected on the surfaces of penile intraepithelial neoplasia (PeIN),[21,22] penile cancer,[23,24] genital warts and other EGLs,[25] and skin warts.[26] Some EGLs such as PeIN are precursors of penile cancer.[85] Less prevalent in the United States,[28] penile carcinoma constitutes up to 10% of all cancers among men in low-resource countries,[29] and its incidence is on the rise in some European countries.[30,31,86] Genital warts (condyloma acuminata) are a common sexually transmitted infection (STI). Although, benign lesions, genital warts cause considerable psychological discomfort and treatment related burden to patients.[87]. Both genital warts and PeIN (low-grade and high-grade) are associated with an increased risk for carcinoma of the penis.[33,34]

The role of cutaneous HPV in the etiology of SCC of the skin and the detection of cutaneous HPV DNA on the surfaces of EGLs suggest a role in the pathogenesis of the squamous epithelium lesions both in the UV exposed and unexposed areas of the body. However, little data exist on the epidemiology and

serology of cutaneous HPV and their distributions and etiologic role in the development of EGLs. Previously we detected β-HPV DNA on 61.1% of all EGLs.[25] Subsequently, in a nested case-control study *(Campbell et al. under review)* we detected a lower prevalence of β-HPV DNA on EGLs compared to controls, and found that some β types were negatively associated with condyloma. To our knowledge, no study has yet prospectively examined the association of seropositivity to cutaneous HPV types and the risk of EGLs in men. The purpose of this study was to examine the association between seropositivity to cutaneous HPV types and the risk of subsequent development of EGLs among men in a case-control study nested in the *HIM* cohort.

METHODS

Study Population

This nested case-control study evaluated 163 incident EGL cases and 352 EGL-free controls in the *HIM* cohort, a multinational prospective study of the natural history of HPV infection in men. Study population and methods have been described previously in detail.[53,54] Briefly, between July 2005 and September 2009, *the HIM Study* enrolled over 4000 men in Brazil, Mexico and the U.S. Participants were eligible if they were: male, aged 18-70 years at baseline, reported no previous diagnosis of penile or anal cancer, had no previous diagnoses of ano-genital warts, no current history or treatment for sexually transmitted infections including HIV, and no current discharges from the penis or burning sensation during urination. Participants were followed every six months for a median of four years. At each study visit, participants completed a computer-assisted self-interviewed questionnaire provided urine and blood samples, and underwent a clinical examination.

Cases included pathologically confirmed incident EGLs ascertained through visual inspection and confirmed with pathology following biopsy. The *HIM* S*tudy* pathology protocol was implemented between February 2009 and May 2013. Approximately 2,754 men, who had ≥2 study visits approximately six months apart were included in the biopsy sub-cohort, described previously in detail.[88,89] Briefly, at each

visit, a trained clinician examined men under 3x light magnification for EGLs. Using shave excision, a tissue specimen was collected from each lesion. Excised tissues were placed in 10% buffered formalin, sent to the University of South Florida Dermatopathology Laboratory and evaluated by two independent pathologists. Discrepant diagnoses were adjudicated by a panel of three independent pathologists and quality control was conducted on 10% of all biopsy specimens.[88] Based on previously described criteria,[88,90] EGLs were categorized as 'condyloma', 'suggestive of condyloma', 'PeIN' and 'EGLs other than condyloma, suggestive of condyloma and PeIN' (i.e. the other EGL cases). A lesion with koilocytes, papillomatosis, hypergranulosis, parakeratosis and dilated blood vessels was diagnosed as 'condyloma', and a lesion without koilocytes but with one or two of the other features associated with a condyloma was considered 'suggestive of condyloma'. These lesions were most likely early condyloma that did not manifest complete histological features of a fully developed condyloma. 'Condyloma' and 'suggestive of condyloma' were therefore combined in the statistical analyses (i.e. the combined condyloma group). Lesions such as molluscum contagiosum, intradermal nevus, fibroepithelial polyp (skin tag), chronic balanitis, genital melanotic macule, psoriasiform dermatitis, lichenoid tissue reaction, and acute mucositis, which had unknown etiology on gross examination, but presented definite histology on pathologic review, were categorized into the 'other EGL cases' group. Finally, lesions with pre-neoplastic or neoplastic cells were categorized as 'PeIN'. Additionally, all four categories of pathological diagnoses were combined into one group 'all EGL cases' to achieve a large sample size during multivariate modeling.

Controls included *the HIM Study* participants who did not develop an EGL throughout the entire four years of follow-up. Two controls, frequency matched on the length of follow up, were selected per case. All study procedures were approved by the Institutional Review Boards of the University of South Florida (Tampa, FL, U.S.), the Ludwig Institute for Cancer Research (Sao Paulo, Brazil), the Centro de Referencia e Terinamento em Doencas Sexualmente Transmissiveis e AIDS (Sao Paulo, Brazil), and the Instituto Nacional de Salud Publica de Mexico (Cuernavaca, Mexico).

Specimens and Data Collection

At each study visit, *HIM Study* participants provided detailed information on demographics (age, race, ethnicity, education and marital status), socioeconomic status, medical history, smoking habits, alcohol consumption, and sexual history. Archived biopsy cohort baseline serum specimens among both cases and controls were tested for seroreactivity to the L1 protein of 14 cutaneous HPV types including β types (5, 8, 12, 14, 17, 22, 23, 24, 38, and 47), α type 27, γ type 4, μ type 1, and ν type 41. Only 14 types were tested due to limited funds, and were carefully selected based on previous reports of their association with cutaneous SCC[19,48] and detection on the surface of EGLs.[25] The antibody detection method was based on a glutathione S-transferase capture enzyme-linked immunosorbent assay (ELISA), in combination with fluorescent bead technology (Luminex) as previously described.[55,56,91] To define type-specific HPV seropositivity, standard cut-off values (200 median fluorescence intensity [MFI]) were applied, as previously described.[57,58]

Statistical Analysis

Demographic, lifestyle and sexual behavior characteristics were compared between cases and controls using Fisher's exact tests for categorical variables (Table 3.1). Type-specific seroprevalence was defined as the proportion of men who tested seropositive for antibodies to a given HPV type. Seroprevalence to any cutaneous HPV (any-HPV) was defined as the proportion of men who were seropositive to at least one of the 14 types of HPV included in this study, and seroprevalence to any-β HPV was defined as the proportion of men who were seropositive to at least one of the 10 β-HPV types. Type-specific and grouped seroprevalence was calculated for different diagnostic categories of EGLs and controls. Seropositivity to one type, two types, and ≥3 types was also estimated. To assess for confounding, associations between factors listed in Table 1 and any-HPV, any-β, and type-specific HPV seroprevalence were examined among controls; associations between these factors and EGL development were assessed among the seronegative groups (unexposed) for any-HPV, any-β, and type-specific HPV. Also, factors associated with the cutaneous HPV seroprevalence, reported previously for the same population (Chapter

2), were considered for inclusion in the multivariate modeling. The final multivariate model included country, age, education, circumcision status, number of lifetime female sex partners, and number of lifetime male sex partners. Using these adjustment factors and logistic regression, separate models were estimated for associations between seropositivity to any-HPV type, any-β HPV type, and each specific HPV type, and EGL development. Interaction with seropositivity to nine mucosal HPV vaccine types (6, 11, 16, 18, 31, 33, 45, 52 and 58.) was also assessed. Additionally, it was hypothesized that cutaneous HPV seropositivity could possibly be associated with condyloma that have definite HPV 6/11 etiology. Therefore, stratified associations were evaluated in the condyloma group by tissue DNA positivity to HPV 6/11 and antibody status to HPV 6/11. All analyses were performed in SAS 9.3.

RESULTS

The characteristics of the 163 incident EGL cases and 352 controls are shown in Table 3.1. Among 48 men diagnosed with other EGL cases, statistically significant differences ($p<0.05$) by country, race, ethnicity, education, marital status, and circumcision were observed when compared to controls. Men from Brazil and Mexico, the "other" race group, Hispanics, men with ≤12 years of education, and uncircumcised men were more likely to have other EGLs diagnoses compared with controls. In total, 109 men were diagnosed with condyloma (n=62) or suggestive of condyloma (n=47). Significant differences by the number of recent female sex partners among those reporting ever having a female sex partner were observed for the combined group of condyloma where men with ≥2 recent female sex partners were more likely to have condyloma compared to controls. Six men were diagnosed with PeIN. Significant differences between PeIN and controls were not observed for any of the socio-demographic and behavioral characteristics evaluated.

Table 3.2 presents seropositivity of type-specific and grouped cutaneous HPV by case-control status. Approximately, 58.3% and 37.5% of men in the other EGL cases group, 71.6% and 46.8% in the combined condyloma, 66.8% and 50.0% in the PeIN, and 71.9% and 38.4% in the control group were seropositive for any-HPV and any-β HPV, respectively. The most commonly occurring type-specific HPV

were ϰ-HPV 4, μ-HPV 1, and β-HPV 8. Approximately 25.0% of men in the other EGL cases group, 31.2% in the combined condyloma, 50.0% in the PeIN, and 31.0% in the control group were seropositive for ϰ-HPV 4. Approximately 14.6% and 22.9% of men in the other EGL cases group, 25.7% and 22.9% in the combined condyloma, and 33.3% and 33.3% in the PeIN, and 31.3% and 19.6% in the control group were seropositive for μ-HPV 1 and β-HPV 8, respectively. Seropositivity to only one HPV type ranged from (16.7%) to (35.5%), to two HPV types from (10.4%) to (20.2%), and to ≥3 HPV types from (22.0%) to (33.3%) across different pathologic diagnoses.

The univariate and multivariate associations of grouped and type-specific cutaneous HPV seropositivity and each of three case outcomes (all EGL cases, combined condyloma, and other EGL cases) are presented in Table 3.3. No association was observed between any-HPV, any-β, and type-specific HPV seropositivity and 'all EGL' cases in either the univariate or the multivariate analyses, except for μ-HPV 1. Seropositivity to μ-HPV 1 was inversely associated with 'all EGL' cases in the unadjusted model (odds ratio [OR] 0.65; 95% confidence interval [CI]: 0.42–0.99). Similarly, no association was observed between any-HPV, any-β, and type-specific HPV seropositivity and 'combined condyloma' and 'other EGL' cases in either the univariate or the multivariate analyses, except for μ-HPV 1. Seropositivity to μ-HPV 1 was inversely associated with other EGL cases (OR 0.38; 95% CI: 0.16 – 0.86) in unadjusted analyses, but failed to reach statistical significance in the adjusted models.

In multivariate analyses a non-significant pattern of elevated risk was observed for any-β HPV and α-HPV type 27 seropositivity in all categories of EGLs compared to controls [(AOR 1.46; 95%CI: 0.95–2.24) and (AOR 1.43; 95% CI: 0.75–2.75) for all EGL cases, [(AOR 1.53; 95% CI: 0.95–2.47) and (AOR 1.24; 95% CI: 0.58–2.62) for combined condyloma] and [(AOR 1.23; 95% CI: 0.59–2.56) and (AOR 2.31; 95% CI: 0.88–6.05) for other EGLs cases], respectively. No significant association was observed when the association between grouped and type-specific cutaneous HPV seropositivity was examined with separate categories of condyloma, and suggestive of condyloma compared to controls (Table A2.4 in Appendices). Furthermore, no significant association was observed between grouped and type-specific cutaneous HPV seropositivity and condyloma when condyloma were stratified by tissue HPV 6/11 DNA positivity or

antibody status to HPV 6 /11 (Table A2.5 in Appendices). Out of the 109 combined condyloma cases examined in this study, 71 (65.1%) were tissues positive for HPV 6/11 DNA, and 21 (19.3%) men with condyloma were also seropositive for HPV 6/11. No significant interaction by seropositivity to ≥1 nine vaccine type HPV was observed (Table A2.6 in Appendices); therefore, the interaction terms were removed from the final models.

DISCUSSION

This is the first study to examine the association between cutaneous HPV seropositivity and EGLs in a prospective, multi-national cohort of men. Overall, the seroprevalence of grouped and type-specific HPV was similar across different EGL categories and controls with the most frequent types being ɤ-HPV 4, μ-HPV 1, and β-HPV 8. We did not observe an association between any-HPV, any-β HPV, and type-specific HPV seropositivity and different pathological diagnoses of EGLs [i) other EGL cases, ii) combined condyloma, and iii) all EGLs cases]. However, a non-significant pattern of elevated risk was observed for any-β HPV and α-HPV type 27 seropositivity in all categories of EGLs compared to controls. In contrast, a non-significant pattern of reduced risk was observed for μ-HPV 1 seropositivity in all categories of EGLs.

The null findings in this study suggest that cutaneous HPV is not associated with EGLs in contrast to previous studies of other cutaneous lesions. Reasons for these differences may include anatomic location of the lesions (i.e. UV exposed versus unexposed), immune status of the participants, and different high-risk groups of populations as previously reported.[92] The first evidence of the cutaneous HPV association with the development of skin lesions was observed among immunosuppressed individuals.[93,94] Patients with a rare genetic disorder, epidermodysplasia verruciformis (EV) developed HPV-associated keratotic skin lesions primarily on sun-exposed skin with potential for malignant transformation.[92] Later, the role of cutaneous HPV pathogenesis was assessed in organ-transplant recipients who were at high risk for skin warts, mainly on the sun-exposed areas that could carry potential for malignant transformation.[95,96] Although the precise role of cutaneous HPV in the development of squamous cell carcinoma (SCC) is debated, a number of epidemiologic studies have examined the association between grouped and type-

specific cutaneous HPV seropositivity and SCC,[18,19,48-50,57,97,98] and several studies have reported the presence of antibodies against ≥1 genus β HPV types to be associated with the risk of SCC.[19,49,50] The null association in our study is in contrast with the positive associations reported for cutaneous HPV seropositivity and SCC of the skin.

It is hypothesized that cutaneous HPV infection might modify the effect of UV-induced DNA damage and apoptosis leading to accumulation of mutations and cancer initiation.[99] If UV exposure is necessary for cutaneous HPV carcinogenicity, lesions on the genital skin do not have significant UV exposure; therefore, development of EGLs may not be associated with exposure to cutaneous HPV infections.

Different studies have reported a variety of individual types of cutaneous HPV to be associated with the SCC of the skin.[18,19,48,57] Some of this variation in the type-specific associations across studies may be explained in part due to differences in the assays used, study population, age distribution, and cut-points used for seropositivity. However, it is also suggested that unlike mucosal HPV type 16 which is consistently causally associated with various forms of cancers, cutaneous HPV may act as a group of similar viruses with possible oncogenic properties.[48] Contrary to skin cancer studies[20,48,50] that have reported grouped cutaneous HPV association with SCC of the skin particularly for beta genotypes, in our study, seropositivity to grouped cutaneous HPV was not associated with subsequent risk of EGLs.

Cutaneous HPV DNA is abundantly found on skin surfaces and persists in hair follicles of healthy individuals.[100] This ubiquity may largely reflect viral colonization. Also, prevalence based on DNA detection varies considerably between various types of specimens collected from skin, e.g. skin swab vs. biopsy, making prevalence based on DNA detection a less precise measure. Serologic studies measuring antibodies against type-specific L1 major capsid protein of HPV provide evidence of host immune response to previous or recent infection and indicate cumulative exposure to HPV over time. Prevalence based on serology is also not dependent on the anatomic site of HPV infection. However, there are two important limitations to HPV serology studies. All infected individuals do not develop antibodies against HPV infection and antibodies may wane over time. Also, in this study we included only 14 types of cutaneous

HPV, out of which 10 were from genus beta. In contrast, studies on skin cancer generally have included more cutaneous HPV types from genus beta.[19,48,101] This difference may partially explain the grouped cutaneous HPV associations observed in the studies on skin cancer.

In this work a non-significant pattern of reduced risk of EGLs for some HPV types was observed. Previously a few studies have also reported a protective effect for some cutaneous HPV types against skin lesions. For example, Masini et al.[98] reported a protective effect for β-HPV 15 seropositivity against SCC of the skin (OR 0.40; 95% CI: 0.20–0.90). In a recent study by Pierce-Campbell et al. in the same *HIM Study* population, men with condyloma were significantly less likely to have beta-HPV 24 and 47 detected on the surface of the lesion than controls were to have beta-HPV 24 and 47 on the normal genital skin (OR: 0.09, 95% CI: 0.01–0.60 and OR: 0.20, 95% CI: 0.02–0.87, respectively). *(Pierce Campbell et al. under review)*.

High seroprevalence estimates in both cases and controls in the current study may suggest that cutaneous HPV are ubiquitous on skin and that cutaneous HPV might be a commensal component of the microbiological flora of the skin.[61,62] It is also possible that cutaneous HPV might be competing against less prevalent and high risk mucosal HPV types and some types might be involved in a protective role in the skin flora. There is a growing body of literature suggesting that non-oncogenic HPV might be stimulating or inhibiting a co-existing oncogenic HPV through interference or immune cross-reaction.[102,103] However, all these proposed hypotheses need rigorous investigations before meaningful conclusions can be drawn. We also examined the association of grouped and type-specific cutaneous HPV seropositivity and condyloma with definite HPV etiology, categorizing condyloma by tissue DNA positivity to HPV 6/11, and antibody status to HPV 6 /11, but no association was observed.

The current study is an extension of our previous work[25]*(Campbell et al. under review)* examining the role of cutaneous HPV in the development of external genital lesions in men. Biopsy-confirmed incident EGLs, prospective evaluation of the association, and the use of a previously validated[55,101] highly sensitive assay to measure antibodies against L1 cutaneous HPV major capsid protein, are the strengths of this study. Also, important cutaneous HPV types were included in this study based on previous reports of their

associations with SCC,[19,48] and recently their detection on the surface of EGLs.[25] There are also some limitations to this study. The small sample size of the PeIN (n=6) limited our ability to further conduct subgroup and type-specific analyses for PeIN. Antibodies against cutaneous HPV are considered a marker of lifetime exposure to HPV, however, baseline serostatus in our study may have been affected by the seroconversion rate or waning of antibodies over time. Data on the serodynamics of cutaneous HPV do not exist to allow us to evaluate the effect of differential seroconversion rates and waning of antibodies over time in EGL cases and controls. Also, the anatomic site of HPV infection cannot be determined, a limitation relevant to all serological studies.

Our findings show that cutaneous HPV are not associated with the development of EGLs. Further research is needed to understand the natural history and seroepidemiology of HPV infections and their role in the development of skin lesions among men, utilizing longitudinal study designs with repeated measures.

Table 3.1. Association between demographic, lifestyle and sexual behavior factors and external genital lesions (EGLs) and controls

Characteristics	EGLs Other than Conlyloma, Suggestive of Condyloma and PeIN[b] (N=48)			Condyloma and Suggestive of Condyloma (N=109)			PeIN[e] (N=6)			Control (N=352)	
	n	(%)	p	n	%	p	n	%	p	n	%
Country											
U.S.	1	2.1	**<0.001**	32	29.4	0.193	1	16.7	0.275	137	38.9
Brazil	19	39.6		45	41.3		4	66.7		124	35.2
Mexico	28	58.3		32	29.4		1	16.7		91	25.9
Age, Years											
18-30	13	27.1	0.264	50	45.9	0.235	3	50.0	0.496	135	38.4
31-44	26	54.2		45	41.3		3	50.0		151	42.9
45-73	9	18.8		14	12.8		0	0.0		66	18.8
Race											
White	11	22.9	**<0.001**	60	55.0	0.160	4	66.7	0.135	182	51.7
Black	9	18.8		15	13.8		1	16.7		65	18.5
Asian	0	0.0		1	0.9		0	0.0		15	4.3
Native Hawaiian/Pacific Islanders	0	0.0		1	0.9		0	0.0		0	0.0
American Indian, Alaska Native	1	2.1		2	1.8		1	16.7		3	0.9
Others	27	56.3		29	26.6		0	0.0		79	22.4
Not reported	0	0.0		1	0.9		0	0.0		8	2.3
Ethnicity											
Hispanic	34	70.8	**0.002**	46	42.2	0.922	1	16.7	0.478	142	40.3
Non-Hispanic	13	27.1		63	57.8		5	83.3		207	58.8
Not reported	1	2.1		0	0.0		0	0.0		3	0.9
Education, Years											
≤12	27	56.3	**0.028**	49	45.0	0.416	4	66.7	0.681	148	42.0
13-15	6	12.5		27	24.8		1	16.7		99	28.1
≥16	12	25.0		33	30.3		1	16.7		98	27.8
Not reported	3	6.3		0	0.0		0	0.0		7	2.0
Marital Status											
Single/never married	9	18.8	**0.003**	53	48.6	0.076	2	33.3	0.264	139	39.5
Married	23	47.9		28	25.7		2	33.3		132	37.5
Cohabitating, living together	14	29.2		15	13.8		0	0.0		48	13.6
Divorced/separated/widowed	2	4.2		12	11.0		2	33.3		33	9.4
Not reported	0	0.0		1	0.9		0	0.0		0	0.0
Smoking Status											
Current	12	25.0	0.979	35	32.1	0.253	2	33.3	0.061	85	24.1
Former	7	14.6		14	12.8		3	50.0		49	13.9
Never	29	60.4		60	55.0		1	16.7		218	61.9
Alcohol, No. of Drinks/Month											
0	13	27.1	0.637	17	15.6	0.061	2	33.3	0.762	87	24.7
1–30	27	56.3		51	46.8		3	50.0		171	48.6
31-60	4	8.3		12	11.0		1	16.7		38	10.8
≥61	4	8.3		29	26.6		0	0.0		54	15.3
Not reported	0	0.0		0	0.0		0	0.0		2	0.6
Circumcision											
No	40	83.3	**0.001**	69	63.3	0.347	5	83.3	0.407	205	58.2
Yes	8	16.7		40	36.7		1	16.7		147	41.8
Sexual Orientation											
MSW	40	83.3	0.489	94	86.2	0.385	6	100.0	0.470	310	88.1
MSM	5	10.4		10	9.2		0	0.0		29	8.2
MSWM	2	4.2		5	4.6		0	0.0		8	2.3
Missing	1	2.1		0	0.0		0	0.0		5	1.4
No. of Female LTP											
0	3	6.3	0.334	10	9.2	0.618	0	0.0	0.464	36	10.2
1-3	5	10.4		14	12.8		0	0.0		57	16.2
4-18	27	56.3		44	40.4		2	33.3		146	41.5
≥19	11	22.9		35	32.1		4	66.7		103	29.3
Not reported	2	4.2		6	5.5		0	0.0		10	2.8

Continued on next page

Table 3.1. (Continued)

Characteristics	EGLs Other than Conlyloma, Suggestive of Condyloma and PeIN[b] (N=48)			Condyloma and Suggestive of Condyloma (N=109)			PeIN[e] (N=6)			Control (N=352)	
	n	(%)	p	n	%	p	n	%	p	n	%
No. of Male Anal LTP											
0	41	85.4	0.726	91	83.5	0.162	6	100.0	0.764	295	83.8
1-3	1	2.1		3	2.8		0	0.0		11	3.1
≥4	3	6.3		9	8.3		0	0.0		13	3.7
Not reported	3	6.3		6	5.5		0	0.0		33	9.4
Recent Female Sex Partner, #[a]											
0	6	12.5	0.696	14	12.8	**0.043**	0	0.0	0.443	46	14.6
1	25	52.1		39	35.9		3	50.0		170	53.8
≥2	14	29.2		44	40.4		3	50.0		94	29.8
Not reported	3	6.3		12	11.0		0	0.0		6	1.9
Recent Male Sex Partners, # [a]											
0	42	87.5	0.332	91	83.5	0.343	6	100.0	0.836	308	88.5
1	3	6.3		4	3.7		0	0.0		8	2.3
≥2	1	2.1		6	5.5		0	0.0		9	2.6
Not reported	2	4.2		8	7.3		0	0.0		23	6.6

P = p-values compares respective EGL category with the last column (control group). P-values were calculated using Chi-square and Fisher exact tests, significant p-values are highlighted in bold.

EGLs = external genital lesions; PeIN = penile intraepithelial neoplasia; LTP = lifetime sex partners

a. Number of female sex partners in the past 6 months among those reporting ever having a female sex partner; number of male sex partners in the past 6 months among those reporting ever having a male sex partner.

b. This category is also referred to as 'other EGL cases' included different other diagnoses i.e. molluscum contagiosum, intradermal nevus, fibroepithelial polyp (skin tag), chronic balanitis, genital melanotic macule, psoriasiform dermatitis, lichenoid tissue reaction, and acute mucositis.

c. Condyloma: A lesion with koilocytes, papillomatosis, hypergranulosis, parakeratosis and dilated blood vessels.

d. Suggestive of condyloma: A lesion without koilocytes but with one or two of the other features associated with a condyloma. These lesions were most likely early condyloma that did not show complete histological features of a fully developed condyloma.

e. PeIN: A lesion with pre-neoplastic or neoplastic cells.

Table 3.2. Grouped and type-specific cutaneous HPV seropositivity by case-control status

HPV type	EGLs[a] Other than Condyloma, Suggestive of Condyloma and PeIN (N=48)		Condyloma and Suggestive of Condyloma (N=109)[b,c]		PeIN[d] (N=6)		Controls (N=352)	
	n	%	n	%	n	%	n	%
Any-HPV								
Seropositive	28	58.3	78	71.6	4	66.8	253	71.9
Any β HPV								
Seropositive	18	37.5	51	46.8	3	50.0	135	38.4
α-HPV 27								
Seropositive	8	16.7	13	11.9	0	0.0	34	9.7
γ-HPV 4								
Seropositive	12	25.0	34	31.2	3	50.0	109	31.0
μ-HPV 1								
Seropositive	7	14.6	28	25.7	2	33.3	110	31.3
ν-HPV 41								
Seropositive	4	8.3	10	9.2	1	16.7	42	11.9
β-HPV 5								
Seropositive	3	6.3	9	8.3	1	16.7	32	9.1
β-HPV 8								
Seropositive	11	22.9	25	22.9	2	33.3	69	19.6
β-HPV 12								
Seropositive	4	8.3	5	4.6	1	16.7	20	5.7
β-HPV 14								
Seropositive	3	6.3	2	1.8	1	16.7	17	4.8
β-HPV 17								
Seropositive	6	12.5	20	18.4	2	33.3	55	15.6
β-HPV 22								
Seropositive	3	6.3	9	8.3	1	16.7	21	6.0
β-HPV 23								
Seropositive	4	8.3	15	13.8	0	0.0	36	10.2
β-HPV 24								
Seropositive	1	2.1	5	4.6	1	16.7	17	4.8
β-HPV 38								
Seropositive	8	16.7	14	12.8	0	0.0	46	13.1
β-HPV 47								
Seropositive	4	8.3	13	11.9	1	16.7	51	14.5
Seropositivity to 1, 2, ≥ 3 HPV types								
Seropositive to 1 HPV	12	25.0	32	29.4	1	16.7	125	35.5
Seropositive to 2 HPV	5	10.4	22	20.2	1	16.7	57	16.2
Seropositive to ≥ 3 HPV	11	22.9	24	22.0	2	33.3	71	20.2

EGLs = external genital lesions; PeIN = penile intraepithelial neoplasia

a. This category is also referred to as 'other EGL cases' included different other diagnoses i.e. molluscum contagiosum, intradermal nevus, fibroepithelial polyp (skin tag), chronic balanitis, genital melanotic macule, psoriasiform dermatitis, lichenoid tissue reaction, and acute mucositis.

b. Condyloma: A lesion with koilocytes, papillomatosis, hypergranulosis, parakeratosis and dilated blood vessels.

c. Suggestive of condyloma: A lesion without koilocytes but with one or two of the other features associated with a condyloma. These lesions were most likely early condyloma that did not show complete histological features of a fully developed condyloma.

d. PeIN: A lesion with pre-neoplastic or neoplastic cells.

Table 3.3. Association between grouped and type-specific cutaneous HPV seropositivity and EGLs[a]

HPV type	EGLs Other than Condyloma Suggestive of Condyloma and PeIN[b]		Condyloma and Suggestive of Condyloma[c]		All EGL Cases[d]	
	OR	AOR[e] 95% CI	OR	AOR[e] 95% CI	OR	AOR[e] 95% CI
Any-HPV						
Negative	1.00	1.00	1.00	1.00	1.00	1.00
Positive	0.55	0.63 (0.30 - 1.32)	0.98	1.03 (0.61 - 1.72)	0.81	0.91 (0.58 - 1.42)
Any β HPV						
Negative	1.00	1.00	1.00	1.00	1.00	1.00
Positive	0.96	1.23 (0.59 - 2.56)	1.41	1.53 (0.95 - 2.47)	1.27	1.46 (0.95 - 2.24)
α-HPV 27						
Negative	1.00	1.00	1.00	1.00	1.00	1.00
Positive	1.87	2.31 (0.88 - 6.05)	1.27	1.24 (0.58 - 2.62)	1.38	1.43 (0.75 - 2.75)
γ-HPV 4						
Negative	1.00	1.00	1.00	1.00	1.00	1.00
Positive	0.74	0.69 (0.31 - 1.54)	1.01	1.04 (0.63 - 1.72)	0.96	1.00 (0.64 - 1.57)
μ-HPV 1						
Negative	1.00	1.00	1.00	1.00	1.00	1.00
Positive	**0.38**	0.62 (0.23 - 1.65)	0.76	0.79 (0.46 - 1.37)	**0.65**	0.78 (0.48 - 1.28)
ν-HPV 41						
Negative	1.00	1.00	1.00	1.00	1.00	1.00
Positive	0.67	0.78 (0.25 - 2.47)	0.75	0.67 (0.31 - 1.45)	0.75	0.74 (0.38 - 1.44)
β-HPV 5						
Negative	1.00	1.00	1.00	1.00	1.00	1.00
Positive	0.67	1.01 (0.27 - 3.79)	0.90	1.02 (0.45 - 2.3)	0.87	1.02 (0.49 - 2.11)
β-HPV 8						
Negative	1.00	1.00	1.00	1.00	1.00	1.00
Positive	1.22	1.50 (0.64 - 3.50)	1.22	1.55 (0.88 - 2.76)	1.25	1.62 (0.98 - 2.69)
β-HPV 12						
Negative	1.00	1.00	1.00	1.00	1.00	1.00
Positive	1.51	1.41 (0.40 - 4.97)	0.80	0.60 (0.19 - 1.92)	1.08	0.90 (0.37 - 2.18)
β-HPV 14						
Negative	1.00	1.00	1.00	1.00	1.00	1.00
Positive	1.31	1.60 (0.38 - 6.72)	0.37	0.19 (0.02 - 1.53)	0.75	0.64 (0.21 - 1.94)
β-HPV 17						
Negative	1.00	1.00	1.00	1.00	1.00	1.00
Positive	0.77	0.80 (0.28 - 2.30)	1.21	1.20 (0.65 - 2.23)	1.12	1.12 (0.64 - 1.94)
β-HPV 22						
Negative	1.00	1.00	1.00	1.00	1.00	1.00
Positive	1.05	1.52 (0.37 - 6.13)	1.42	1.50 (0.61 - 3.68)	1.37	1.50 (0.67 - 3.35)
β-HPV 23						
Negative	1.00	1.00	1.00	1.00	1.00	1.00
Positive	0.80	1.14 (0.35 - 3.74)	1.40	1.27 (0.63 - 2.57)	1.16	1.10 (0.57 - 2.12)
β-HPV 24						
Negative	1.00	1.00	1.00	1.00	1.00	1.00
Positive	0.42	0.46 (0.05 - 4.14)	0.95	0.96 (0.32 - 2.86)	0.88	0.91 (0.34 - 2.44)
β-HPV 38						
Negative	1.00	1.00	1.00	1.00	1.00	1.00
Positive	1.33	1.82 (0.71 - 4.69)	0.98	0.82 (0.40 - 1.66)	1.04	0.96 (0.52 - 1.76)
β-HPV 47						
Negative	1.00	1.00	1.00	1.00	1.00	1.00
Positive	0.54	0.66 (0.20 - 2.14)	0.80	0.88 (0.44 - 1.79)	0.73	0.85 (0.45 - 1.61)
Seropositivity to 1, 2, ≥ 3 HPV						
Negative	1.00	1.00	1.00	1.00	1.00	1.
Positive to 1 HPV	0.48	0.50 (0.21 - 1.22)	0.82	0.82 (0.45 – 1.51)	0.67	0.71 (0.42 – 1.20)
Positive to 2 HPV	0.43	0.33 (0.08 - 1.30)	1.23	1.37 (0.70 – 2.75)	0.92	1.08 (0.56 – 2.03)
Positive to ≥ 3 HPV	0.77	1.23 (0.47 - 3.21)	1.08	1.12 (0.57 – 2.23)	0.97	1.21 (0.67 – 2.17)

EGLs = external genital lesions; PeIN = penile intraepithelial neoplasia; OR = odds ratios unadjusted; AOR = adjusted odds ratios

a. Due to fewer cases unadjusted and adjusted models were not estimated for PeIN. **b.** This category is also referred as 'other EGL cases' included different other diagnoses i.e. molluscum contagiosum, intradermal nevus, fibroepithelial polyp (skin tag), chronic balanitis, genital melanotic macule, psoriasiform dermatitis, lichenoid tissue reaction, and acute mucositis**. c.**This category included condyloma and suggestive of condyloma. Condyloma: A lesion with koilocytes, papillomatosis, hypergranulosis, parakeratosis and dilated blood vessels. Suggestive of condyloma: A lesion without koilocytes but with one or two of the other features associated with a condyloma. These lesions were most likely early condylomas that did not show complete histological features of a fully developed condyloma. **d.** This category included all pathological diagnoses of EGLs **e**. Odds ratios were adjusted for Country of residence, age, education, circumcision, female lifetime sex partners, and male lifetime sex partners.

CHAPTER FOUR:

MANUSCRIPT 3: SEROPREVALENCE AND ASSOCIATED FACTORS OF NINE VACCINE TYPE HUMAN PAPILLOMAVIRUSES (HPV) AMONG MEN IN THE MULTINATIONAL HIM STUDY

ABSTRACT

Human papillomavirus (HPV) is one of the most common sexually transmitted infections worldwide. Recently a 9-valent HPV (9vHPV) prophylactic vaccine was licensed. Seroprevalence prior to vaccine dissemination is needed for monitoring vaccine effectiveness over time. Few studies have assessed the seroprevalence of 9vHPV types in men. This study investigated the seroprevalence of 9vHPV vaccine types and associated risk factors among men residing in Brazil, Mexico, and the United States. Six hundred men were randomly selected from *the HPV Infection in Men* (*HIM) Study*. Archived serum specimens collected at enrollment were tested for antibodies against nine HPV types (6, 11, 16, 18, 31, 33, 45, 52 and 58) using a GST L1-based multiplex serologic assay. Socio-demographic, lifestyle and sexual behavior data at enrollment were collected through a questionnaire. Binomial proportions were used to estimate seroprevalence and logistic regression was used to examine factors associated with seropositivity.

Overall, 28.3% of men were seropositive for at least one of the 9vHPV vaccine types, 14.0% for at least one of the seven high-risk types (16, 18, 31, 33, 45, 52 and 58) and 11.2% for at least one of the five high-risk types (31, 33, 45, 52 and 58) not included in the quadrivalent HPV vaccine, and 17.4% for at least one of the low-risk types (6/11). In multivariate analyses, compared to men with no anal sex lifetime partners, men with ≥2 partners were more likely to be seropositive for grouped HPV [(9vHPV: adjusted odds ratio (AOR) 2.52; 95% confidence interval (CI) 1.40-4.54), (high-risk HPV: AOR 2.18; 95%CI: 1.05-4.50) and (low-risk HPV: AOR 2.12; 95%CI: 1.12-4.03)], and individual HPV types 6, 16, 33 and 58 with

AORs ranging from 2.19 to 7.36. Compared to men aged 18-30 years, men older than 30 years were significantly more likely to be seropositive for any high-risk HPV, in addition to individual types 18 and 45; and compared to never smokers, current smokers were more likely to be seropositive to 9vHPV, low-risk HPV and HPV 6. In contrast, married men were less likely to be seropositive to any high-risk HPV and individual HPV types 18 and 31 when compared to single men. In conclusion, these data indicate that exposure to the nine HPV types included in the 9vHPV vaccine is common in men and that seropositivity to 9vHPV vaccine types is associated with older age and the lifetime number of anal sex partners.

INTRODUCTION

Human papillomavirus (HPV) is one of the most common sexually transmitted infections (STI) worldwide. Nearly all sexually active adults will be infected with one or more HPV types at some point in their lives.[10,27] More than 40 HPV types are known to infect the ano-genital region, of which at least 13 are classified as oncogenic.[27,104] HPV infection is often asymptomatic and transient; however, a small proportion of infections persist and cause benign and malignant diseases in men and women.[4,105] Oncogenic HPV is a necessary cause of cervical cancer and nearly all cervical, approximately 50% of vulvar, and 65% of vaginal cancers, are caused by oncogenic HPV.[10,14] HPV also causes approximately 40-50% of penile, 85% of anal, 60-70% of oropharyngeal, and 10% of laryngeal cancers.[27,28,106-108] Non-oncogenic HPV types cause genital warts and other benign lesions of epithelial tissues.[11]

In the United States (U.S.), the bivalent and 4-valent HPV vaccines are recommended for routine vaccination for girls at age 11 or 12 years, females age 13 through 26 years, and males age 13 through 21 years if they have not yet received the HPV vaccine.[35] Recently, the Advisory Committee on Immunization Practices (ACIP) added the 9-valent HPV vaccine (9vHPV) to the vaccine recommendations.[36] Information on the natural history of HPV infection and serology in men prior to vaccine dissemination is needed to monitor the effectiveness of the vaccine over time.

Although a number of studies have reported seroprevalences of HPV types 6, 11, 16 and 18 in men from different countries,[37-42] few have examined other HPV types (e.g. 31, 33, 45, 52 and 58) included in

the 9vHPV vaccine in the general population. One study included only men who have sex with men (MSM),[109] another study included men only from the U.S. with an age range of 14-59 years,[110] and two studies using the same study population in the Netherlands examined only the seven high-risk types in the 9vHPV vaccine.[111,112] Previously, we reported seroprevalences for the 4-valent HPV vaccine types (6, 11, 16 and 18).[39] In this study, we extended our analysis to estimate the seroprevalence of 9vHPV vaccine types and investigated factors associated with the seropositivity in men from three countries (Brazil, Mexico, and the U.S.) across a broad age range to report data across the entire adult lifespan and old age.

METHODS

Study Population

Study participants included a sub-cohort of 600 men randomly selected from *the HPV Infection in Men (HIM) Study*. Simple random sampling method was used to select the sub-set because performing serology testing for the entire parent cohort was not viable and cost-effective. The *HIM Study* uses a prospective cohort design to examine the natural history of HPV infections among men in three countries. Detailed descriptions of the study population and procedures have been published elsewhere.[53,54] Briefly, over 4000 men aged 18-70 years at baseline were recruited from Tampa, Florida, Sao Paulo, Brazil, and Cuernavaca, Mexico. Eligibility criteria included no previous history of penile cancer, anal cancer, ano-genital warts and HIV; no current history or treatment for STIs; no current discharges from the penis or burning sensation during urination; and no history of prior or current participation in HPV vaccine traials. Participants were followed every six months for a median of four years. At baseline and each study visit, participants completed a computer-assisted self-interviewed questionnaire, provided blood and urine specimens, and underwent a clinical examination. A total of 3,695 *HIM Study* participants who provided serum specimens and completed the questionnaire at baseline were eligible to be included in the current study. This study was approved by the Institutional Review Boards of the University of South Florida (Tampa, FL, the U.S.), the Ludwig Institute for Cancer Research (Sao Paulo, Brazil), the Centro de

Referencia e Treinamento em Doencas Sexualmente Transmissiveis e AIDS (Sao Paulo, Brazil), and the Instituto Nacional de Salud Publica de Mexico (Cuernavaca, Mexico).

Specimens and Data Collection

At the baseline visit, *the HIM Study* participants provided detailed information on sociodemographic characteristics, smoking habits, alcohol consumption, medical history, and an extensive sexual behaviors history. Archived baseline serum specimens from 600 participants were tested for seroreactivity to L1 major capsid proteins of the 9vHPV vaccine types (6, 11, 16, 18, 31, 33, 45, 52 and 58). The antibody detection method was based on a glutathione S-transferase (GST) capture enzyme-linked immunosorbent assay (ELISA), in combination with fluorescent bead technology previously described.[55,56] To define seropositivity for each of the 9vHPV vaccine types, type-specific cut-off values measured in median fluorescence intensity [MFI] units were applied, as previously described.[56]

Statistical Analysis

Type-specific seroprevalence was calculated as the proportion of men who tested positive for a given HPV type. Four additional categories of HPV (i.e. 9vHPV, high-risk HPV, 5-additional HPV, and low-risk HPV) were also used as dependent variables in the analyses. '9vHPV' seroprevalence was defined as the proportion of men who were seropositive for at least one of the nine vaccine types. 'High-risk HPV' included men who were seropositive for at least one of the seven oncogenic types (i.e. 16, 18, 31, 33, 45, 52 and 58), 'five additional HPV' included men who were seropositive for at least one of the five oncogenic types not included in the quadrivalent HPV vaccine (i.e. 31, 33, 45, 52 and 58), and 'low-risk HPV' included men who were seropositive for HPV types 6 or 11. Two subjects with inadequate serology results were excluded from all analyses resulting in a final sample size of 598 men. Baseline sociodemographic and behavioral factors listed in Table 1 were compared between seropositive and seronegative men with high- and low-risk HPV, and with the full *HIM Study* cohort (>4000 men) using Chi-square tests. Seroprevalence estimates were calculated for each HPV type, by country and age group, and compared using Chi-square

and Fisher's exact tests. The Holm–Bonferroni correction was used to adjust for multiple comparisons when comparing seroprevalence estimates across countries. To examine associations between individual and grouped HPV seropositivity and potential risk factors, logistic regression was used and odds ratios (ORs) and their 95% confidence intervals (CI) were estimated. Factors listed in Table 1 were considered for inclusion in multivariate logistic regression models. Variables were selected using backward stepwise elimination with significance at $p \leq 0.1$. Country and age were forced into the model due to the study design. To assess the individual contribution of each variable retained in the model at $p \leq 0.1$, the likelihood ratio test at $p < 0.05$ was performed. The final multivariate models for 9vHPV and low-risk HPV included country, age, smoking status, and the number of male anal sex lifetime partners (LTP). The final model for high-risk HPV included country, age, marital status, and the number of male anal sex LTP. Multivariate models for individual high-risk HPV types 16, 18, 31, 33, 45, 52 and 58 were adjusted for the same factors as the grouped high-risk HPV model, and multivariate models for individual low-risk HPV types 6 and 11 were adjusted for the same factors as the grouped low-risk HPV model. All analyses were performed in SAS 9.3.

RESULTS

This study included 184 participants from the U.S., 200 from Brazil, and 214 from Mexico. Participant characteristics by seropositivity to high- and low-risk HPV are presented in Table 4.1. Significant differences by age, sexual orientation, lifetime number of anal sex partners (LTP), and recent male anal sex partners were observed for seropositivity to high-risk HPV ($p < 0.05$). Mid-adult men (35-44 years) and older men (45-73 years), men who have sex with men (MSM), men who have sex with men and women (MSMW), men with ≥2 lifetime male anal sex partners (LTP), and men with ≥2 recent anal sex partners were more likely to be seropositive for high-risk HPV ($p < 0.05$). Significant differences in seropositivity to low-risk HPV were observed by country, circumcision status, sexual orientation, and number of male anal sex LTP ($p < 0.05$). Participants in the randomly selected sub-cohort (n=600) were similar ($P > 0.05$) to the full *HIM* cohort (>4,000 men) on all baseline sociodemographic and sexual behavioral characteristics listed in Table 1 (Table A2.1 in Appendices).

Grouped and individual type HPV seroprevalence estimates are presented in Table 4.2. Overall, 28.3% of men were seropositive for ≥1 9vHPV type, 14.0% for ≥1 high-risk HPV types, 11.2% for ≥1 of the five additional types, and 17.4% for low-risk HPV types 6 and/or 11. Type-specific seropositivity for five individual HPV types was as follows: HPV 31 (4.5%), HPV 33 (1.7%), HPV 45 (3.7%), HPV 52 (2.5%), and HPV 58 (4.7%). Seropositivity for 9vHPV was statistically significantly different by country [Brazil (36.5%), Mexico (28.5%) and U.S. (19.0%); overall $p<0.001$] and remained significant only for Brazil vs. U.S. ($p<0.001$) after pair wise comparisons. No significant differences in the seroprevalence of grouped high-risk HPV types was observed by country [Brazil (14.5%), Mexico (16.8%) and U.S. (10.3%); overall $p=0.173$]. Seroprevalence of the five additional types also did not vary significantly by country [Brazil (10.5%) and Mexico (13.6) and U.S. (9.2%); overall $p=0.368$]. Seropositivity to low-risk HPV was statistically significantly different by country [Brazil (27.5%), Mexico (14.0%) and U.S. (10.3%); overall $p<0.001$] and remained significant for Brazil vs. U.S. ($p<0.001$) and Brazil vs. Mexico ($p<0.001$) after pair wise comparisons. Seroprevalence of individual HPV types 6 and 16 significantly differed by country [Brazil (25.5%) and (5.5%), Mexico (13.6%) and (1.9%) and U.S. (9.2%) and (1.6%), respectively; overall p-values<0.05]. After pair wise comparisons, significant difference remained for Brazil vs. U.S. ($p<0.001$) and Brazil vs. Mexico ($p=0.004$) only for HPV 6. Seroprevalence was highest for HPV 6 in all three countries [Brazil (25.5%), Mexico (13.6%) and U.S. (9.2%)]. Figures 4.1A and B show grouped and type-specific HPV seroprevalence by age group. A significant positive trend ($p_{trend} <0.05$) for seropositivity with increasing age was observed for grouped high-risk HPV, grouped five additional types, and some individual HPV types (18, 45 and 58).

Factors associated with seropositivity of grouped and specific individual HPV types are presented in Tables 4.3A-C. In multivariable analyses, adjusting for all other variables in the table, number of anal sex lifetime partners, was significantly associated with seropositivity for grouped and specific individual HPV types (6, 16, 33, and 58). Compared to men with no reported anal sex, men with ≥2 anal sex LTP were more likely to be seropositive for grouped HPV [(9vHPV: adjusted odds ratio (AOR) 2.52; 95%CI: 1.40-4.54), (high-risk HPV: AOR 2.18; 95%CI: 1.05-4.50) and (low-risk HPV: AOR 2.12; 95%CI: 1.12-4.03)]

respectively. Similarly, men with ≥2 anal sex LTP were significantly more likely to be seropositive for individual HPV types 6, 16, 33 and 58, with AORs ranging from 2.19 to 7.36. Increasing age was significantly positively associated with seropositivity for high-risk HPV and HPV types 18 and 45. Compared to men aged 18-30 years, men aged 31-44 years and men aged 45-73 were more likely to be seropositive for high-risk HPV [(AOR 1.95; 95%CI: 1.03-3.70) and (AOR 2.75; 95%CI: 1.23-6.15), respectively] and HPV 18 [(AOR 4.60; 95%CI: 1.24-17.10) and (AOR 14.47; 95%CI: 3.24-64.68) respectively]. Compared to men aged 18-30 years, men aged 45-73 years were more likely to be seropositive for HPV 45 (AOR 5.43; 95%CI: 1.27-23.25). Country was significantly associated with seropositivity for grouped and individual HPV types. Compared to men from the U.S., men from Brazil were more likely to be seropositive to 9vHPV (AOR 2.18; 95%CI: 1.31-3.60), low-risk HPV (AOR 3.13; 95%CI: 1.71-5.75), HPV 6 (AOR 3.15; 95% CI: 1.67-5.93), HPV 11 (AOR 3.43; 95%CI: 1.04-11.35), and less likely to be seropositive to HPV 58 (AOR 0.12; 95%CI: 0.03-0.53). Similarly, compared to men from the U.S., men from Mexico were more likely to be seropositive for 9vHPV (AOR 1.66; 95%CI: 1.02-2.71), high-risk HPV (AOR 2.24; 95%CI: 1.15-4.40) and HPV 18 (AOR 7.91; 95%CI: 1.51-41.34). Compared to never smokers, current smokers were more likely to be seropositive to 9v-HPV, low-risk HPV and HPV 6. In contrast, compared to men who reported being single/never married, married/cohabiting men were less likely to be seropositive to high-risk HPV, HPV types 18 and 31 (Table 4.3A-C).

DISCUSSION

Previously, we reported seroprevalence of the 4-valent HPV vaccine types (6, 11, 16 and 18).[39] In this study, we extended our analysis to include five additional HPV types (31, 33, 45, 52 and 58) in the 9vHPV vaccine. Additionally, we investigated factors associated with 9vHPV seropositivity in 598 men recruited from Brazil, Mexico and the U.S. across a broad age range (18-73 years).

Over 28.3% of men were seropositive for ≥1 9vHPV vaccine types, and 11.2% for ≥ 1 of the five additional high-risk types. Type specific seropositivity for five additional types was as follows: [HPV 31 (4.5%), 33 (1.7%), 45 (3.7%), 52 (2.5%), and 58 (4.7%)]. Age, lifetime number of male anal sex partners,

and smoking were positively associated with seropositivity of grouped and individual HPV types. A recent study[110] used competitive Luminex Immunoassay (cLIA) and estimated seroprevalence of 9vHPV vaccine types among men using the National Health and Nutrition Examination Survey (NHANES) data. The seroprevalence estimates for 9vHPV, and 5-additional types in our study were higher than the NHANES study [(28.3% vs. 19.4%) and (11.2% vs. 6.6%) respectively]. The type-specific seroprevalence estimates of the five additional types were also slightly higher in our study [HPV 31 (4.5% vs. 2.3%), 33 (1.7% vs. 1.6%), 45 (3.7% vs. 0.7%), 52 (2.5% vs. 1.3%), and 58 (4.7% vs. 1.5%). Another study[112] estimated the seroprevalence of five additional types in the Netherlands using virus-like particles (VLP) based serologic assay and reported slightly higher seroprevalence estimates than our study for HPV types 33 (1.7% vs. 6.0%), 45 (3.7% vs. 6.8%) and 52 (2.5% vs.5.2%); and slightly lower estimates for types 31 (4.5% vs. 2.5%) and 58 (4.7% vs. 3.7%). Some of this variation could be partially explained by the differences in serologic assays used, study population, and age distribution across studies.

Seroprevalence estimates of grouped and individual 4-valent HPV vaccine types in our study were considerably lower than the estimates we had previously reported using VLP-based serologic assay.[39] Important demographic and sexual behavior confounders such as age, marital status, education, and lifetime number of anal sex partners were similar in both current and previous studies, except for country of residence. Compared to previous study, current study had slightly fewer men from the U.S. (31% vs. 37%) and marginally higher from Brazil (33 vs. 30%). Since men from U.S. are less likely, and men from Brazil are more likely, to be seropositive for 4-valent HPV types as reported in both studies, this difference in the sample distribution by country of residence is not likely to underestimate the seroprevalence estimates in the current study compared to our previous reports. We believe that this variation is due to differences in the serologic assays used in our current and previous study, and that the GST L1-based multiplex serologic assay underestimated seroprevalence estimates for 4vHPV types in the current study. Nevertheless, both studies have consistently reported that men with multiple lifetime number of anal sex partner, older age, single men, and Brazilians are more likely to be seropositive for 4-valent HPV types.

The positive association of increasing age and high-risk HPV in this study is consistent with previous serology studies in men,[37,39,113,114] and this may reflect the fact that exposure to HPV and antibodies as a response accumulate over time. The positive association of the increasing number of male anal sex LTP with the seropositivity of HPV is also consistent with previous studies,[39,115] and is likely to be explained by i) the increased probability of exposure to HPV types due to multiple sex partners, ii) the highest DNA prevalence of HPV infection and persistence in the anal canal of MSM, and iii) the anatomic site-specific immune response. Studies have shown that HPV infection is common in the anal canal, and its prevalence is higher among MSM than men who have sex with women (MSW).[115-117] Recently, emerging evidence showed that HPV infection persists longer in the anal canal of MSM[118] which may explain the highest incidence of HPV-related anal cancers in MSM.[119] Also, it is suggested that the viral antigen is more efficiently detected by the immune system in the mucosal epithelium. Therefore, mucosal epithelium (e.g. anal canal) is more likely to induce immune response to HPV infections compared to keratinized epithelium (e.g. penile skin).[119-121] Therefore, MSM are more likely to have incident, prevalent, and persistent HPV infections, and potentially may induce greater immune response because of the anatomic location of their infection compared to other sub-groups (e.g. MSW) resulting in high seroprevalence. Current smokers were more likely to be seropositive to low-risk HPV (AOR 1.96; 95%CI: 1.17-3.29). Syrjanen et al.[122] and Dunne et al.[37] also reported a two-fold increased risk of low-risk HPV (6/11) seropositivity for current male smokers. However, Liu et al.[110] did not find an association between smoking and low-risk HPV. In contrast, married/cohabiting men were less likely to be seropositive to high-risk HPV compared to single men. Similar associations have also been reported by other studies previously.[112,123,124] This association is likely to be partially explained that single men might have more opportunity of meeting new sex partners than married men, therefore, they have a high probability of exposure to different HPV types.

Inclusion of men from three countries across a broad range of ages, detailed information on demographic and sexual history, and the estimation of prevalence based on type-specific antibodies against L1 HPV capsid protein in a single laboratory with one protocol are the strengths of this study. Some limitations should be considered when interpreting the results. This study was a cross-sectional analysis

which utilized serology and risk factor data collected at baseline. HPV prevalence based on antibody response may be affected by waning of antibodies over time and by the seroconversion rate since all infected individuals do not produce an immune response to HPV infections; therefore, prevalence and cumulative exposure of HPV might be underestimated. Seropositivity in our study was measured by GST-based multiplex serologic assay and may not be comparable to estimates measured using other assays. Risk factor data was self-reported. Due to social desirability and recall biases lifetime number of sex partners may be under- or over-reported. However, the use of computer-assisted self-interviews for sexual behavior data may have minimized the social desirability bias. Despite these limitations, this study provides important data on the distribution of 9-valent HPV vaccine types in a sample of men from three countries.

In summary, more than a quarter of men were seropositive for at least one of the nine vaccine type HPV, and MSM were two and half times more likely to be seropositivity to ≥1 HPV types, indicating the need for HPV immunization in men, and particularly among MSM.

Table 4.1. Participant characteristics by seropositivity to high-risk and low-risk types in 9vHPV vaccine

	High-risk HPV (16, 18, 31, 33, 45, 52, 58) Seropositive			Low-risk HPV (6, 11) Seropositive		
Characteristic	**n**	**%**	**p-value[a]**	**n**	**%**	**p-value[a]**
Country						
U.S.	19	10.3	0.173	19	10.3	**<0.001**
Brazil	29	14.5		55	27.5	
Mexico	36	16.8		30	14.0	
Age, Years						
18-30	26	10.0	**0.018**	46	17.8	0.976
31-44	40	15.6		44	17.2	
45-73	18	21.7		14	16.9	
Race						
White	44	16.4	0.248	56	20.8	0.229
Black	6	7.7		11	14.1	
Asian	2	11.8		2	11.8	
American Indian/Alaska Native	0	0.0		3	33.3	
Other	30	13.8		32	14.7	
Ethnicity						
Hispanic	42	14.9	0.535	45	16.0	0.383
Non-Hispanic	40	13.1		57	18.7	
Education, Years						
≤12	45	15.7	0.104	54	18.8	0.572
13-15	14	8.8		28	17.6	
≥16	23	15.4		22	14.8	
Marital Status						
Single/never married	35	13.5	0.140	52	20.1	0.232
Married/cohabitating	35	12.6		44	15.8	
Divorced/separated/widowed	13	22.4		7	12.1	
Not reported	1	33.3		1	33.3	
Smoking Status						
Never	47	13.9	0.909	50	14.7	0.104
Former	15	13.3		21	18.6	
Current	22	15.1		33	22.6	
Alcohol, No. Drinks/Month						
0	18	12.8	0.818	31	22.0	0.124
1–30	41	14.7		40	14.3	
31-60	10	17.2		14	24.1	
≥61	14	12.7		18	16.4	
Circumcision						
No	57	14.7	0.561	77	19.8	**0.034**
Yes	27	12.9		27	12.9	
Sexual Orientation						
MSW	67	12.8	**0.011**	79	15.1	**0.001**
MSM	12	23.5		17	33.3	
MSWM	5	33.3		7	46.7	
No. of Female LTP						
0	7	11.7	0.663	15	25.0	0.372
1-3	21	16.7		20	15.9	
4-18	32	13.0		45	18.3	
≥19	15	11.9		19	15.1	
No. of Male Anal Sex LTP						
0	67	13.2	**0.003**	76	15.0	**0.001**
1	0	0.0		7	25.0	
≥2	15	25.9		20	34.5	
Recent Female Sex Partners, #[b]						
0	22	19.1	0.096	14	12.2	0.111
1	26	10.8		36	14.9	
≥2	24	14.8		34	21.0	
Recent Male Anal Sex Partners, #[b]						
0	7	12.7	**0.021**	14	25.4	0.256
1	1	7.7		6	46.2	
≥2	8	38.1		8	38.1	

[a] Chi-square and Fisher's exact tests were used to calculate p-values. significant p-value is marked in bold

b. Number of female/male sex partners in the past 6 months among those reporting ever having a female/male sex partner

Note: LTP = lifetime partners, MSW=men who have sex with women; MSM=men who have sex with men; MSWM=men who have sex with women and men

Table 4.2. Grouped and type-specific seroprevalence of 9vHPV types among men from Brazil, Mexico and the U.S.

	Overall (N=598)	U.S. (N=184)	Brazil (N=200)	Mexico (N=214)	
HPV Type	**%**	**%**	**%**	**%**	**P-value[e]**
HPV 6	16.2	9.2	25.5	13.6	**<0.001**
HPV 11	3.8	2.2	6.0	3.3	0.129
HPV 16	3.0	1.6	5.5	1.9	**0.041**
HPV 18	3.5	1.1	4.0	5.1	0.082
HPV 31	4.5	3.8	4.0	5.6	0.628
HPV 33	1.7	1.6	2.0	1.4	0.892
HPV 45	3.7	1.6	5.0	4.2	0.189
HPV 52	2.5	3.3	1.5	2.8	0.513
HPV 58	4.7	6.5	2.0	5.6	0.081
9vHPV[a]	28.3	19.0	36.5	28.5	**0.001**
High-risk (16, 18, 31, 33, 45, 52, 58)[b]	14.0	10.3	14.5	16.8	0.173
Five additional types (31, 33, 45, 52, 58)	11.2	9.2	10.5	13.6	0.368
Low-risk (6, 11)[c]	17.4	10.3	27.5	14.0	**<0.001**
4vHPV types (6, 11, 16, 18)[d]	21.2	12.5	32.0	18.7	**<0.001**

[a.] 9vHPV category included nine vaccine types (6, 11, 16, 18, 31, 33, 45, 52, and 58). '9vHPV' variable was created, if a man was seropositive for one or more of the 9vHPV vaccine types then the '9vHPV' variable was seropositive otherwise seronegative.
[b.] High-risk category included seropositivity to at least one of the seven types of HPV (16, 18, 31, 33, 45, 52, and 58).
[c.] Low-risk category included seropositivity to at least one of the two types of HPV (6 and 11).
[d.] 4vHPV category included seropositivity to at least one of the four types of HPV (6, 11, 16, and 18).
[e]. P-values were calculated by Chi-square and Fisher exact tests. Significant p-value is marked in bold.

Table 4.3A. Factors independently associated with seropositivity of nine vaccine type HPV in men from Brazil, Mexico and the U.S.

Characteristics	9vHPV[a]		High Risk HPV[b]		Low Risk HPV[c]		HPV 6	
	OR (95%CI)	AOR (95%CI)[e]	OR (95%CI)	AOR (95%CI)[e]	OR (95%CI)	AOR (95%CI)[e]	OR (95%CI)	AOR (95%CI)[e]
Country								
U.S.	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Brazil	**2.45**	**2.18 (1.31 - 3.60)**	1.47	1.17 (0.59 - 2.35)	**3.29**	**3.13 (1.71 - 5.75)**	**3.36**	**3.15 (1.67 - 5.93)**
Mexico	**1.70**	**1.66 (1.02 - 2.71)**	1.76	**2.24 (1.15 - 4.40)**	1.42	1.39 (0.74 - 2.62)	1.54	1.48 (0.77 - 2.86)
Age, Years								
18-30	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
31-44	1.12	0.95 (0.63 - 1.43)	1.66	**1.95 (1.03 - 3.70)**	0.96	0.78 (0.48 - 1.26)	0.99	0.79 (0.48 - 1.30)
45-73	1.62	1.43 (0.81 - 2.51)	**2.48**	**2.75 (1.23 - 6.15)**	0.94	0.83 (0.41 - 1.68)	1.05	0.92 (0.45 - 1.89)
Marital Status								
Single or never married	--	--	1.00	1.00	--	--	--	--
Married/cohabitating	--	--	1.02	**0.50 (0.26 - 0.99)**	--	--	--	--
Divorced/separated/widowed	--	--	1.15	1.18 (0.52 - 2.70)	--	--	--	--
Smoking Status								
Never	1.00	1.00	--	--	1.00	1.00	1.00	1.00
Current	1.51	**1.57 (1.01 - 2.44)**	--	--	**1.69**	**1.96 (1.17 - 3.29)**	**1.83**	**2.12 (1.25 - 3.61)**
Former	1.14	1.14 (0.69 - 1.89)	--	--	1.32	1.52 (0.84 - 2.77)	1.41	1.58 (0.85 - 2.91)
No. of Male Anal Sex LTP								
0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
1	0.97	0.78 (0.32 - 1.92)	NE	NE	1.89	1.37 (0.54 - 3.44)	2.08	1.52 (0.6 - 3.83)
≥2	**3.11**	**2.52 (1.40 - 4.54)**	**2.29**	**2.18 (1.05 - 4.50)**	**2.98**	**2.12 (1.12 - 4.03)**	**3.04**	**2.19 (1.14 - 4.21)**

OR = unadjusted odds ratio; AOR = adjusted odds ratio; CI = 95% confidence interval; LTP = lifetime partners

'--' variable not included in the final adjusted model

[a.] This category included seropositivity to ≥1 of the nine vaccine types (6, 11, 16, 18, 31, 33, 45, 52, 58)

[b.] This category included seropositivity to ≥ 1of the high-risk types in the vaccine (16, 18, 31, 33, 45, 52, 58)

[c.] This category included seropositivity to ≥ 1 of the low-risk types in the vaccine (16, 18)

[e.] Final adjusted models were estimated using backward stepwise elimination method with a significance level of p-value ≤ 0.1 for retention. Odds ratios were adjusted for all other variables in the column.

Table 4.3B. Factors independently associated with seropositivity of nine vaccine type HPV in men from Brazil, Mexico and the U.S.

Characteristics	HPV 11		HPV 16		HPV 18		HPV 31	
	OR (95%CI)	AOR (95%CI)[e]	OR (95%CI)	AOR (95%CI)[e]	OR (95%CI)	AOR (95%CI)[e]	OR (95%CI)	AOR (95%CI)[e]
Country								
U.S.	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Brazil	2.87	**3.43 (1.04 - 11.35)**	3.51	1.71 (0.4 - 7.39)	3.79	3.22 (0.61 - 16.97)	1.05	1.08 (0.34 - 3.42)
Mexico	1.52	1.66 (0.47 - 5.91)	1.15	1.43 (0.29 - 7.10)	**4.93**	**7.91 (1.51 - 41.34)**	1.50	2.65 (0.93 - 7.58)
Age, Years								
18-30	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
31-44	0.73	0.62 (0.24 - 1.60)	1.71	1.52 (0.42 - 5.42)	2.59	**4.60 (1.24 - 17.1)**	0.71	1.08 (0.39 - 2.98)
45-73	1.14	0.98 (0.29 - 3.35)	1.04	0.59 (0.08 - 4.26)	**5.87**	**14.47 (3.24 - 64.68)**	0.66	1.04 (0.24 - 4.52)
Marital Status								
Single or never married	--	--	1.00	1.00	1.00	1.00	1.00	1.00
Married/cohabitating	--	--	0.76	0.66 (0.17 - 2.56)	0.89	**0.17 (0.05 - 0.56)**	0.60	**0.32 (0.10 - 0.99)**
Divorced/separated/widowed	--	--	1.31	3.58 (0.84 - 15.19)	0.37	0.11 (0.01 - 1.00)	0.37	0.82 (0.20 - 3.42)
Smoking Status								
Never	1.00	1.00	--	--	--	--	--	--
Current	1.50	1.73 (0.65 - 4.65)	--	--	--	--	--	--
Former	1.38	1.57 (0.51 - 4.84)	--	--	--	--	--	--
No. of Male Anal Sex LTP								
0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
1	NE	NE	NE	NE	NE	NE	NE	NE
≥2	1.90	1.34 (0.41 - 4.41)	**8.85**	**7.33 (2.22 - 24.17)**	0.50	2.48 (0.78 - 7.93)	1.63	1.72 (0.51 - 5.88)

OR = unadjusted odds ratio; AOR = adjusted odds ratio; CI = 95% confidence interval; LTP = lifetime partners
'--' variable not included in the final adjusted model
e. Final adjusted models were estimated using backward stepwise elimination method with a significance level of p-value ≤ 0.1 for retention. Odds ratios were adjusted for all other variables in the column.

Table 4.3C. Factors independently associated with seropositivity of nine vaccine type HPV in men from Brazil, Mexico and the U.S.

Characteristics	HPV 33		HPV 45		HPV 52		HPV 58	
	OR (95%CI)	AOR (95%CI)[e]	OR (95%CI)	AOR (95%CI)[e]	OR (95%CI)	AOR (95%CI)[e]	OR (95%CI)	AOR (95%CI)[e]
Country								
U.S.	1.00	1.00	1.00	1.00	1.00	1.00	1.00	
Brazil	1.23	0.8 (0.14 - 4.60)	3.17	2.52 (0.62 - 10.28)	0.45	0.30 (0.06 - 1.51)	**0.29**	**0.12 (0.03 - 0.53)**
Mexico	0.86	1.47 (0.26 - 8.26)	2.65	3.05 (0.72 - 12.87)	0.86	0.99 (0.27 - 3.67)	0.85	0.87 (0.33 - 2.29)
Age, Years								
18-30	1.00	1.00	1.00	1.00	1.00	1.00	1.00	
31-44	0.81	0.83 (0.17 - 4.03)	2.28	3.14 (0.94 - 10.51)	0.84	0.63 (0.15 - 2.57)	1.68	1.82 (0.61 - 5.44)
45-73	0.62	0.48 (0.04 - 6.05)	**3.96**	5.43 (1.27 - 23.25)	2.14	1.22 (0.25 - 5.90)	**2.89**	2.90 (0.81 - 10.47)
Marital Status								
Single or never married	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Married/cohabitating	0.20	0.36 (0.06 - 2.35)	1.00	0.38 (0.12 - 1.17)	0.69	1.35 (0.29 - 6.21)	0.98	0.83 (0.26 - 2.62)
Divorced/separated/widowed	1.04	2.05 (0.29 - 14.39)	0.37	0.18 (0.02 - 1.57)	0.76	4.72 (0.90 - 24.77)	1.93	1.80 (0.47 - 6.81)
Smoking Status								
Never	--	--	--	--	--	--	--	--
Current	--	--	--	--	--	--	--	--
Former	--	--	--	--	--	--	--	--
No. of Male Anal Sex LTP								
0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
1	NE	NE	NE	NE	NE	NE	NE	NE
≥2	**6.19**	**7.36 (1.56 - 34.77)**	**2.90**	1.9 (0.59 - 6.14)	2.25	4.03 (0.89 - 18.28)	1.99	**4.49 (1.36 - 14.8)**

OR = unadjusted odds ratio; AOR = adjusted odds ratio; CI = 95% confidence interval; LTP = lifetime partners
'--' variable not included in the final adjusted model
e. Final adjusted models were estimated using backward stepwise elimination method with a significance level of p-value ≤ 0.1 for retention. Odds ratios were adjusted for all other variables in the column.

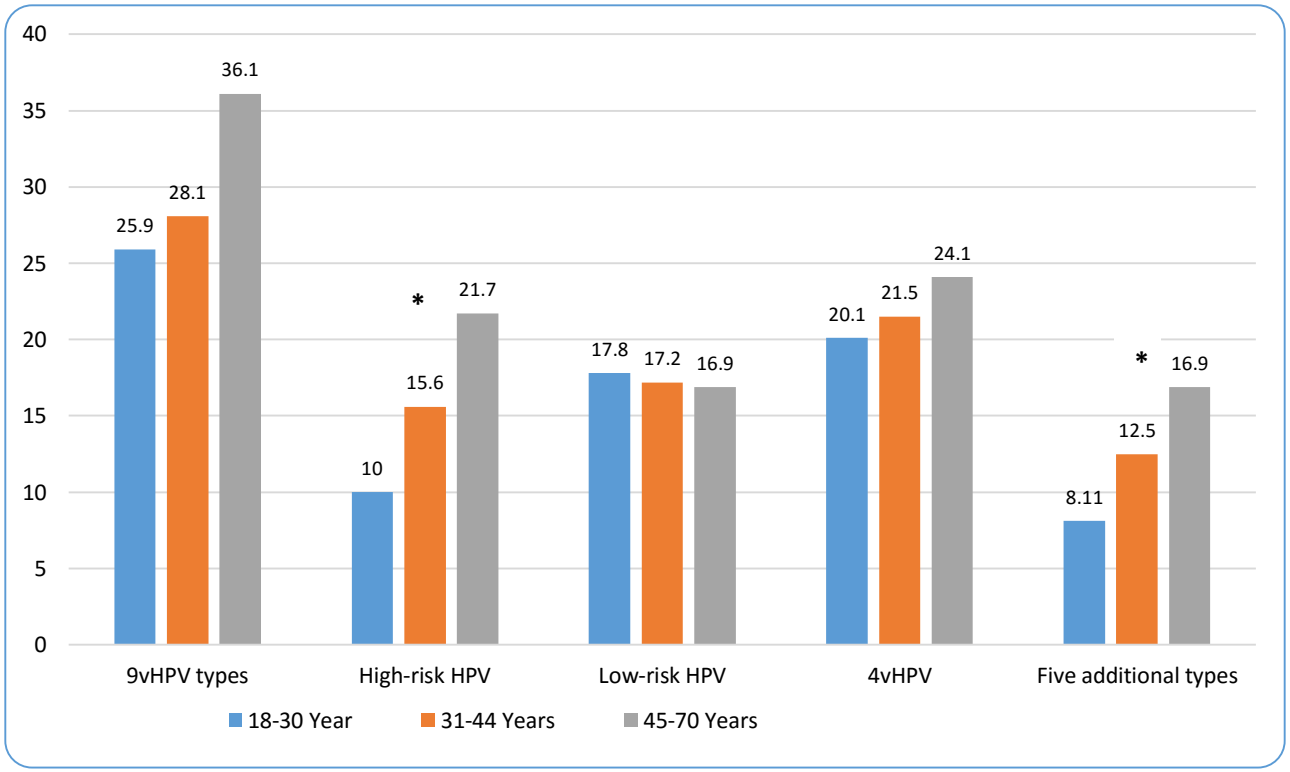

Figure 4.1A. Grouped HPV seroprevalence of nine vaccine types by age group

Figure 4.1A shows seropositivity for 9vHPV, high-risk, five additional types, low-risk, and 4vHPV categories by three age groups (18-30 years, 31-44 years, and 45-73 years). 9vHPV category included nine vaccine types (6, 11, 16, 18, 31, 33, 45, 52 and 58). '9vHPV' variable was created, if a man was seropositive for one or more of the nine vaccine types then '9vHPV' was seropositive otherwise seronegative. 4vHPV category included seropositivity to at least one of the four types of HPV (6, 11, 16 and 18). High risk category included seropositivity to at least one of the seven types of HPV (16, 18, 31, 33, 45, 52 and 58). Low risk category included seropositivity to at least one of the two types of HPV (6 and 11). Significant p-value <0.05 is marked with '*'. P-value was obtained from Cochran-Armitage Trend Test. P_{trend} <0.05 for high-risk and five additional types categories.

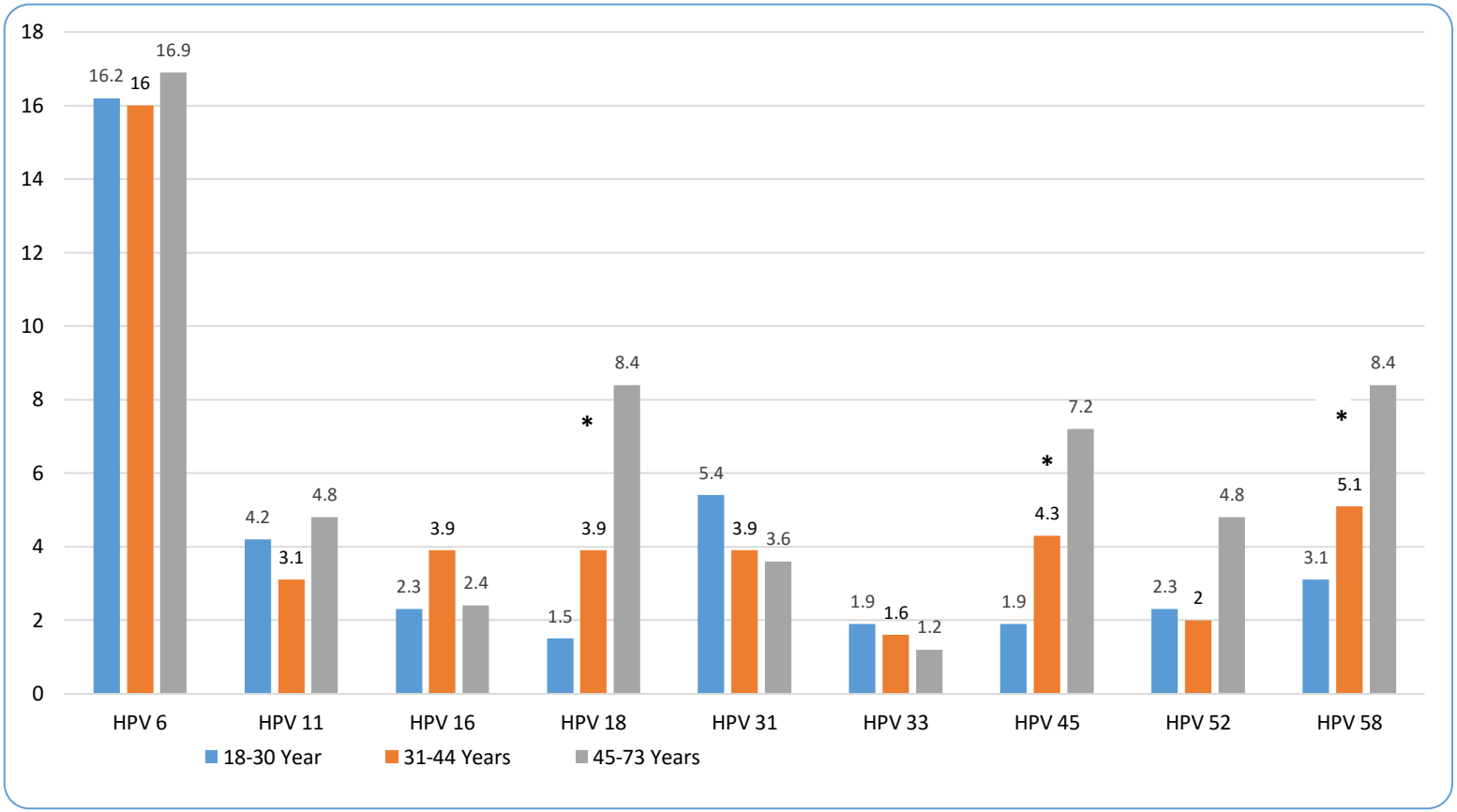

Figure 4.1B. Type-specific seroprevalence of nine HPV vaccine types by age group

Figure 4.1B shows seropositivity for type-specific HPV. Significant p-value <0.05 is marked with '*'. P-value was obtained from Cochran-Armitage Trend Test. P_{trend} <0.05 for HPV types 18, 45 and 58.

CHAPTER FIVE:

CONCLUSIONS AND FUTURE DIRECTIONS

This study estimated the seroprevalence of 14 cutaneous HPV, β types (5, 8, 12, 14, 17, 22, 23, 24, 38 and 47), α type 27, γ type 4, μ type 1, and ν type 41, and 9vHPV vaccine types (6, 11, 16, 18, 33, 45, 52 and 58) and investigated factors associated with the seropositivity in a random sample of 600 men residing in Brazil, Mexico, and the U.S. enrolled in the prospective *HIM Study*. It also investigated association between seropositivity to cutaneous HPV types and the risk of external genital lesions (EGLs) comparing 163 incident EGL cases to 352 EGL-free controls nested in the *HIM* cohort. This was one of the few studies to estimate seroprevalence of cutaneous HPV and 9vHPV types and evaluate association between cutaneous HPV seropositivity and the risk of EGLs prospectively in men across a broad range of age from three countries.

Approximately 65.4% of men were seropositive for ≥1 cutaneous HPV types, and 39.0% for ≥1 β-HPV types. The most commonly occurring type-specific HPV were ϫ-HPV 4, μ-HPV 1, and β-HPV 8. Compared to men with ≤12 years of education, men with ≥16 years of education, and compared to men aged 18-30 years, men aged 31-44 years, were 1.75 times and 1.72 times more likely to be seropositive to ≥1 cutaneous HPV types, respectively. Country of residence was significantly associated with β-HPV types (5, 12, 14, 24 and 47), older age was significantly positively associated with β-HPV types (8, 14, 22, 38 and 47), circumcision status was significantly positively associated with β-HPV types (5, 14, 24 and 47) and γ-HPV type 4, and lifetime number of male anal sex partners were significantly positively associated with HPV types (23, 24 and 17). These data show that exposure to cutaneous HPV is common among men

and that prevalence estimates based on antibodies against L1 major capsid HPV protein represent cumulative exposure to cutaneous HPV over time which may rise with increasing age.

Several studies have reported a positive association between cutaneous HPV seropositivity and skin cancer, and a variety of cutaneous HPV types are detectable in the genital epithelial lesions. However, it remains unclear whether these viruses are causally associated with the diseases. This study also investigated association between seropositivity to cutaneous HPV types and the risk of development of EGLs among men from three countries. Incident EGL cases were ascertained through visual inspection, verified through biopsy and pathological examination, and categorized into condyloma, suggestive of condyloma, penile intraepithelial neoplasia (PeIN), and other EGL cases. Controls included men who did not develop any EGL in the entire follow up period. No statistically significant association was observed between grouped and type-specific cutaneous HPV seropositivity and development of EGLs across all pathological diagnoses. Overall, the seroprevalences of grouped and type-specific HPV were similar across different EGL categories and controls with the most frequent types being ϒ type 4, μ type 1 and β type 8. Our data show that exposure to cutaneous HPV is common, and exposure to cutaneous HPV is not likely to increase the risk of EGLs among men.

Recently, the 9-vHPV vaccine was approved for use in for both in men and women living in the U.S. In addition to the four HPV types (6, 11, 16 and 18) targeted previously in the quadrivalent HPV vaccines, the 9vHPV vaccine also prevents against five additional high-risk HPV types (31, 33, 45, 52 and 58). Measuring seropositivity before vaccine introduction and dissemination provides baseline data on exposure and is essential for monitoring the effectiveness of vaccines over time. This study also estimated seroprevalence of 9vHPV types and investigated factors associated with the seropositivity in a random sample of 600 men enrolled in the *HIM* cohort. Approximately 28.3% of men were seropositive for ≥1 9vHPV types, 14.0% for ≥1 seven high-risk HPV, 11.2% for ≥1 five additional high-risk, and 17.4% for ≥1 low-risk HPV types (6/11). Compared to men with no partners, men with ≥2 lifetime anal sex partners were two times significantly more likely to be seropositive for grouped 9vHPV, high-risk, and low risk

HPV, and individual types (6, 16, 33, and 58) with odds ratios ranging from 2.19 to 7.36. Compared to men aged 18-30 years, men aged >30 years were two times significantly more likely to be seropositivity for grouped high-risk HPV, and compared to never, current smokers were nearly two times more likely to be seropositivity for low-risk HPV. In contrast, compared to single men, married men were two times less likely to be seropositive for grouped high-risk HPV. Our data show that exposure to 9vHPV vaccine types is common among men. Older men and men who have sex with men are at highest risk.

Inclusion of men from three countries across a broad range of age, detailed information on demographic and sexual history, the estimation of prevalence based on antibodies against L1 HPV capsid protein in a single laboratory simultaneously by multiplex serology, biopsy-confirmed incident EGL cases, and the prospective evaluation of the association are major strengths of this study. However, this study has limitations that should be considered when interpreting the results. Antibodies against L1 HPV capsid protein are considered a marker of cumulative exposure to HPV infections. However, all infected men with HPV do not induce antibody response and that the antibodies may decline over time. Therefore, prevalence and cumulative exposure of HPV in our study might be underestimated. Due to lack of information on serodynamics of cutaneous HPV, the impact of seroconversion, clearance of infection, and waning of antibodies over time could not be assessed. Also, the anatomic site of HPV infection cannot be determined. These are limitations relevant to all serological studies. Seropositivity in our study was measured by GST-based multiplex serologic assay and these estimates may not be comparable to estimates measured by other HPV serologic assays. The limited number of PeIN cases restricted our ability to conduct sub-group analyses for PeIN. Despite these limitations, this study provides important data and contributes to the understanding of the distribution and associated factors of cutaneous HPV and 9vHPV. Furthermore, it provides evidence on the etiologic role of exposure to cutaneous HPV in development of EGLs.

This study was one of the few to estimate seroprevalence of cutaneous HPV and 9vHPV types in men across a broad range of age from three different countries and examined the etiologic role of exposure to cutaneous HPV and the risk of EGLs. To validate these results, future large prospective studies of

cutaneous HPV with repeated measures are recommended to evaluate the etiologic role of cutaneous HPV in the development of skin lesions including skin cancer, and benign and malignant diseases of the genitalia. Research on HPV is rapidly evolving and new types and strains are discovered on a regular basis, future studies should include newly discovered and less explored types and sub-types of HPV as well. It is also recommended that more than one measure of HPV exposure should be used and that the impact of seroconversion rate along with the decay of antibodies over time be assessed. Until the current international efforts to standardize HPV serologic assays yield some fruitful results, it is also recommended that future studies utilize more than one serologic assays so that the results could be compared and contrasted to previous literature utilizing different assays. Also, future studies should focus on identifying viral mechanism and pathway of pathogenesis for cutaneous HPV infections which is currently unknown at large. Finally, future research should focus on summarizing and consolidating results from studies on HPV epidemiology, serology, and natural history to inform public health policy and practice and vaccine development efforts.

REFERENCES

1. Riethmuller D, Schaal JP, Mougin C. [Epidemiology and natural history of genital infection by human papillomavirus]. *Gynecologie, obstetrique & fertilite.* 2002;30(2):139-146.

2. Insinga RP, Dasbach EJ, 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(11):1107-1122.

3. Centers for Disease Control and Prevention. Epidemiology and Prevention of Vaccine-Preventable Diseases. Hamborsky J KA, Wolfe S, eds. 13th ed. Washington D.C. Public Health Foundation, 2015.

4. Ault KA. Epidemiology and natural history of human papillomavirus infections in the female genital tract. *Infectious diseases in obstetrics and gynecology.* 2006;2006 Suppl:40470.

5. Clifford GM, Gallus S, Herrero R, et al. Worldwide distribution of human papillomavirus types in cytologically normal women in the International Agency for Research on Cancer HPV prevalence surveys: a pooled analysis. *Lancet.* 2005;366(9490):991-998.

6. Dunne EF, Nielson CM, Stone KM, Markowitz LE, Giuliano AR. Prevalence of HPV infection among men: A systematic review of the literature. *The Journal of infectious diseases.* 2006;194(8):1044-1057.

7. Sichero L, Pierce Campbell CM, Fulp W, et al. High genital prevalence of cutaneous human papillomavirus DNA on male genital skin: the HPV Infection in Men Study. *BMC infectious diseases.* 2014;14:677.

8. Bernard HU, Burk RD, Chen Z, van Doorslaer K, zur Hausen H, de Villiers EM. Classification of papillomaviruses (PVs) based on 189 PV types and proposal of taxonomic amendments. *Virology.* 2010;401(1):70-79.

9. De Villiers E-M, Fauquet C, Broker TR, Bernard H-U, zur Hausen H. Classification of papillomaviruses. *Virology.* 2004;324(1):17-27.

10. Weaver BA. Epidemiology and natural history of genital human papillomavirus infection. *The Journal of the American Osteopathic Association.* 2006;106(3 Suppl 1):S2-8.

11. Trottier H, Franco EL. The epidemiology of genital human papillomavirus infection. *Vaccine.* 2006;24 Suppl 1:S1-15.

12. Struijk L, Bouwes Bavinck JN, Wanningen P, et al. Presence of human papillomavirus DNA in plucked eyebrow hairs is associated with a history of cutaneous squamous cell carcinoma. *J Invest Dermatol.* 2003;121(6):1531-1535.

13. Proby CM, Harwood CA, Neale RE, et al. A case-control study of betapapillomavirus infection and cutaneous squamous cell carcinoma in organ transplant recipients. *Am J Transplant.* 2011;11(7):1498-1508.

14. Walboomers JM, Jacobs MV, Manos MM, et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. *J Pathol.* 1999;189(1):12-19.

15. American Cancer Society. Cancer Facts & Figures 2015. Available at http://www.cancer.org/acs/groups/content/@editorial/documents/document/acspc-044552.pdf. .

16. Chen JG, Fleischer AB, Jr., Smith ED, et al. Cost of nonmelanoma skin cancer treatment in the United States. *Dermatologic surgery : official publication for American Society for Dermatologic Surgery [et al.]*. 2001;27(12):1035-1038.

17. Karia PS, Han J, Schmults CD. Cutaneous squamous cell carcinoma: estimated incidence of disease, nodal metastasis, and deaths from disease in the United States, 2012. *Journal of the American Academy of Dermatology*. 2013;68(6):957-966.

18. Andersson K, Waterboer T, Kirnbauer R, et al. Seroreactivity to cutaneous human papillomaviruses among patients with nonmelanoma skin cancer or benign skin lesions. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology*. 2008;17(1):189-195.

19. Iannacone MR, Gheit T, Waterboer T, et al. Case-control study of cutaneous human papillomaviruses in squamous cell carcinoma of the skin. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology*. 2012;21(8):1303-1313.

20. Karagas MR, Waterboer T, Li Z, et al. Genus beta human papillomaviruses and incidence of basal cell and squamous cell carcinomas of skin: population based case-control study. *Bmj*. 2010;341:c2986.

21. Kreuter A, Brockmeyer NH, Weissenborn SJ, et al. Penile intraepithelial neoplasia is frequent in HIV-positive men with anal dysplasia. *J Invest Dermatol*. 2008;128(9):2316-2324.

22. Wieland U, Jurk S, Weissenborn S, Krieg T, Pfister H, Ritzkowsky A. Erythroplasia of queyrat: coinfection with cutaneous carcinogenic human papillomavirus type 8 and genital papillomaviruses in a carcinoma in situ. *J Invest Dermatol*. 2000;115(3):396-401.

23. Humbey O, Cairey-Remonnay S, Guerrini JS, et al. Detection of the human papillomavirus and analysis of the TP53 polymorphism of exon 4 at codon 72 in penile squamous cell carcinomas. *Eur J Cancer*. 2003;39(5):684-690.

24. Heideman DA, Waterboer T, Pawlita M, et al. Human papillomavirus-16 is the predominant type etiologically involved in penile squamous cell carcinoma. *J Clin Oncol*. 2007;25(29):4550-4556.

25. Pierce Campbell CM, Messina JL, Stoler MH, et al. Cutaneous human papillomavirus types detected on the surface of male external genital lesions: a case series within the HPV Infection in Men Study. *Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology*. 2013;58(4):652-659.

26. Harwood CA, Spink PJ, Surentheran T, et al. Degenerate and nested PCR: a highly sensitive and specific method for detection of human papillomavirus infection in cutaneous warts. *J Clin Microbiol*. 1999;37(11):3545-3555.

27. IARC. Human Papillomavirus. IARC working group on the evaluation of carcinogenic risks to humans. Vol. 90. Lyon, France: International Agency for Research on Cancer, 2007 IARC monographs on the evaluation of carcinogenic risks).

28. Hernandez BY, Barnholtz-Sloan J, German RR, et al. Burden of invasive squamous cell carcinoma of the penis in the United States, 1998-2003. *Cancer*. 2008;113(10 Suppl):2883-2891.

29. Misra S, Chaturvedi A, Misra NC. Penile carcinoma: a challenge for the developing world. *The Lancet. Oncology.* 2004;5(4):240-247.

30. Baldur-Felskov B, Hannibal CG, Munk C, Kjaer SK. Increased incidence of penile cancer and high-grade penile intraepithelial neoplasia in Denmark 1978-2008: a nationwide population-based study. *Cancer causes & control : CCC.* 2012;23(2):273-280.

31. Graafland NM, Verhoeven RH, Coebergh JWW, Horenblas S. Incidence trends and survival of penile squamous cell carcinoma in the Netherlands. *International Journal of Cancer.* 2011;128(2):426-432.

32. Arya M, Li R, Pegler K, et al. Long-term trends in incidence, survival and mortality of primary penile cancer in England. *Cancer Causes & Control.* 2013;24(12):2169-2176.

33. Blomberg M, Friis S, Munk C, Bautz A, Kjaer SK. Genital warts and risk of cancer: a Danish study of nearly 50 000 patients with genital warts. *The Journal of infectious diseases.* 2012;205(10):1544-1553.

34. Cubilla AL, Velazquez EF, Young RH. Epithelial lesions associated with invasive penile squamous cell carcinoma: a pathologic study of 288 cases. *International journal of surgical pathology.* 2004;12(4):351-364.

35. Markowitz LE, Dunne EF, Saraiya M, et al. Human papillomavirus vaccination: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep.* 2014;63(RR-05):1-30.

36. Petrosky E, Bocchini J, Hariri S, et al. Use of 9-valent human papillomavirus (HPV) vaccine: updated HPV vaccination recommendations of the advisory committee on immunization practices. *MMWR Morb Mortal Wkly Rep.* 2015;64(11):300-304.

37. Dunne EF, Nielson CM, Hagensee ME, et al. HPV 6/11, 16, 18 seroprevalence in men in two US cities. *Sex Transm Dis.* 2009;36(11):671-674.

38. Lu B, Hagensee ME, Lee JH, et al. Epidemiologic factors associated with seropositivity to human papillomavirus type 16 and 18 virus-like particles and risk of subsequent infection in men. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology.* 2010;19(2):511-516.

39. Lu B, Viscidi RP, Lee JH, et al. Human papillomavirus (HPV) 6, 11, 16, and 18 seroprevalence is associated with sexual practice and age: results from the multinational HPV Infection in Men Study (HIM Study). *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology.* 2011;20(5):990-1002.

40. Introcaso CE, Dunne EF, Hariri S, Panicker G, Unger ER, Markowitz LE. Prevaccine era human papillomavirus types 6, 11, 16 and 18 seropositivity in the U.S.A., National Health and Nutrition Examination Surveys, 2003-2006. *Sex Transm Infect.* 2014;90(6):505-508.

41. Thompson DL, Douglas JM, Jr., Foster M, et al. Seroepidemiology of infection with human papillomavirus 16, in men and women attending sexually transmitted disease clinics in the United States. *The Journal of infectious diseases.* 2004;190(9):1563-1574.

42. Strickler HD, Kirk GD, Figueroa JP, et al. HPV 16 antibody prevalence in Jamaica and the United States reflects differences in cervical cancer rates. *Int J Cancer.* 1999;80(3):339-344.

43. Zheng ZM, Baker CC. Papillomavirus genome structure, expression, and post-transcriptional regulation. *Front Biosci.* 2006;11:2286-2302.

44. Steben M, Duarte-Franco E. Human papillomavirus infection: epidemiology and pathophysiology. *Gynecol Oncol.* 2007;107(2 Suppl 1):S2-5.

45. Lacey CJ, Lowndes CM, 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.

46. Parkin DM, Bray F. Chapter 2: The burden of HPV-related cancers. *Vaccine.* 2006;24 Suppl 3:S3/11-25.

47. Steben M, Garland SM. Genital warts. *Best Pract Res Clin Obstet Gynaecol.* 2014;28(7):1063-1073.

48. Waterboer T, Abeni D, Sampogna F, et al. Serological association of beta and gamma human papillomaviruses with squamous cell carcinoma of the skin. *The British journal of dermatology.* 2008;159(2):457-459.

49. Feltkamp MC, Broer R, di Summa FM, et al. Seroreactivity to epidermodysplasia verruciformis-related human papillomavirus types is associated with nonmelanoma skin cancer. *Cancer research.* 2003;63(10):2695-2700.

50. Karagas MR, Nelson HH, Sehr P, et al. Human papillomavirus infection and incidence of squamous cell and basal cell carcinomas of the skin. *Journal of the National Cancer Institute.* 2006;98(6):389-395.

51. Struijk L, Hall L, van der Meijden E, et al. Markers of cutaneous human papillomavirus infection in individuals with tumor-free skin, actinic keratoses, and squamous cell carcinoma. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology.* 2006;15(3):529-535.

52. Hampras SS, Giuliano AR, Lin HY, et al. Natural history of cutaneous human papillomavirus (HPV) infection in men: the HIM study. *PLoS One.* 2014;9(9):e104843.

53. 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 epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology.* 2008;17(8):2036-2043.

54. Giuliano AR, Lee JH, Fulp W, et al. Incidence and clearance of genital human papillomavirus infection in men (HIM): a cohort study. *Lancet.* 2011;377(9769):932-940.

55. Sehr P, Muller M, Hopfl R, Widschwendter A, Pawlita M. HPV antibody detection by ELISA with capsid protein L1 fused to glutathione S-transferase. *Journal of virological methods.* 2002;106(1):61-70.

56. Waterboer T, Sehr P, Michael KM, et al. Multiplex human papillomavirus serology based on in situ-purified glutathione s-transferase fusion proteins. *Clin Chem.* 2005;51(10):1845-1853.

57. Casabonne D, Michael KM, Waterboer T, et al. A prospective pilot study of antibodies against human papillomaviruses and cutaneous squamous cell carcinoma nested in the Oxford component of the European Prospective Investigation into Cancer and Nutrition. *Int J Cancer.* 2007;121(8):1862-1868.

58. Michael KM, Waterboer T, Sehr P, et al. Seroprevalence of 34 human papillomavirus types in the German general population. *PLoS pathogens.* 2008;4(6):e1000091.

59. Holm S. A simple sequentially rejective multiple test procedure. *Scandinavian journal of statistics.* 1979:65-70.

60. Iannacone MR, Michael KM, Giuliano AR, Waterboer T, Pawlita M, Rollison DE. Risk factors for cutaneous human papillomavirus seroreactivity among patients undergoing skin cancer screening in Florida. *The Journal of infectious diseases.* 2010;201(5):760-769.

61. Hannigan GD, Grice EA. Microbial ecology of the skin in the era of metagenomics and molecular microbiology. *Cold Spring Harb Perspect Med.* 2013;3(12):a015362.

62. Antonsson A, Forslund O, Ekberg H, Sterner G, Hansson BG. The ubiquity and impressive genomic diversity of human skin papillomaviruses suggest a commensalic nature of these viruses. *J Virol.* 2000;74(24):11636-11641.

63. Bzhalava D, Guan P, Franceschi S, Dillner J, Clifford G. A systematic review of the prevalence of mucosal and cutaneous human papillomavirus types. *Virology.* 2013;445(1-2):224-231.

64. Boxman IL, Russell A, Mulder LH, et al. Association between epidermodysplasia verruciformis-associated human papillomavirus DNA in plucked eyebrow hair and solar keratoses. *J Invest Dermatol.* 2001;117(5):1108-1112.

65. de Koning MN, Weissenborn SJ, Abeni D, et al. Prevalence and associated factors of betapapillomavirus infections in individuals without cutaneous squamous cell carcinoma. *J Gen Virol.* 2009;90(Pt 7):1611-1621.

66. Sampogna F, Bavinck JN, Pawlita M, et al. Factors associated with the seroprevalence of 26 cutaneous and two genital human papillomavirus types in organ transplant patients. *J Gen Virol.* 2012;93(Pt 1):165-174.

67. Albero G, Castellsague X, Lin HY, et al. Male circumcision and the incidence and clearance of genital human papillomavirus (HPV) infection in men: the HPV Infection in men (HIM) cohort study. *BMC infectious diseases.* 2014;14:75.

68. Albero G, Castellsagué X, Giuliano AR, Bosch FX. Male circumcision and genital human papillomavirus: a systematic review and meta-analysis. *Sexually transmitted diseases.* 2012;39(2):104-113.

69. 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. *International Journal of Cancer.* 2009;124(6):1251-1257.

70. Warren J. Physical Effects of Circumcision. *Genital Autonomy:*: Springer; 2010:75-79.

71. Taylor JR, Lockwood AP, Taylor AJ. The prepuce: specialized mucosa of the penis and its loss to circumcision. *British journal of urology.* 1996;77(2):291-295.

72. Ekstrom J, Bzhalava D, Svenback D, Forslund O, Dillner J. High throughput sequencing reveals diversity of Human Papillomaviruses in cutaneous lesions. *Int J Cancer.* 2011;129(11):2643-2650.

73. Bottalico D, Chen Z, Dunne A, et al. The oral cavity contains abundant known and novel human papillomaviruses from the Betapapillomavirus and Gammapapillomavirus genera. *The Journal of infectious diseases.* 2011;204(5):787-792.

74. Farzan SF, Waterboer T, Gui J, et al. Cutaneous alpha, beta and gamma human papillomaviruses in relation to squamous cell carcinoma of the skin: a population-based study. *Int J Cancer.* 2013;133(7):1713-1720.

75. Molano M, Posso H, Weiderpass E, et al. Prevalence and determinants of HPV infection among Colombian women with normal cytology. *British journal of cancer.* 2002;87(3):324-333.

76. Chandra A. *Sexual behavior, sexual attraction, and sexual identity in the United States: data from the 2006-2008 National Survey of Family Growth.* Citeseer; 2011.

77. Hariri S, Dunne EF, Sternberg M, et al. Seroepidemiology of human papillomavirus type 11 in the United States: results from the third National Health And Nutrition Examination Survey, 1991--1994. *Sex Transm Dis.* 2008;35(3):298-303.

78. Svare EI, Kjaer SK, Worm AM, Osterlind A, Meijer CJ, van den Brule AJ. Risk factors for genital HPV DNA in men resemble those found in women: a study of male attendees at a Danish STD clinic. *Sex Transm Infect.* 2002;78(3):215-218.

79. Van Doorslaer K, Tan Q, Xirasagar S, et al. The Papillomavirus Episteme: a central resource for papillomavirus sequence data and analysis. *Nucleic Acids Res.* 2013;41(Database issue):D571-578.

80. Chouhy D, Bolatti EM, Perez GR, Giri AA. Analysis of the genetic diversity and phylogenetic relationships of putative human papillomavirus types. *J Gen Virol.* 2013;94(Pt 11):2480-2488.

81. de Villiers EM, Gunst K. Characterization of seven novel human papillomavirus types isolated from cutaneous tissue, but also present in mucosal lesions. *J Gen Virol.* 2009;90(Pt 8):1999-2004.

82. Proby CM, Harwood CA, Neale RE, et al. A case-control study of betapapillomavirus infection and cutaneous squamous cell carcinoma in organ transplant recipients. *Am J Transplant.* 2011;11(7):1498-1508.

83. Asgari MM, Kiviat NB, Critchlow CW, et al. Detection of human papillomavirus DNA in cutaneous squamous cell carcinoma among immunocompetent individuals. *J Invest Dermatol.* 2008;128(6):1409-1417.

84. Quint KD, Genders RE, de Koning MN, et al. Human Beta-papillomavirus infection and keratinocyte carcinomas. *J Pathol.* 2015;235(2):342-354.

85. IARC. Human papillomaviruses: Iarc Working Group on the Evaluation of Carcinogenic Risks to Humans. 2007;90:1-636.

86. Arya M, Kalsi J, Kelly J, Muneer A. Malignant and premalignant lesions of the penis. *Bmj.* 2013;346:f1149.

87. Insinga RP, Dasbach EJ, 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(11):1397-1403.

88. Ingles DJ, Pierce Campbell CM, Messina JA, et al. Human papillomavirus virus (HPV) genotype- and age-specific analyses of external genital lesions among men in the HPV Infection in Men (HIM) Study. *The Journal of infectious diseases.* 2015;211(7):1060-1067.

89. Sudenga SL, Ingles DJ, Pierce Campbell CM, et al. Genital Human Papillomavirus Infection Progression to External Genital Lesions: The HIM Study. *Eur Urol.* 2015.

90. Anic GM, Messina JL, Stoler MH, et al. Concordance of human papillomavirus types detected on the surface and in the tissue of genital lesions in men. *J Med Virol.* 2013;85(9):1561-1566.

91. Sehr P, Zumbach K, Pawlita M. A generic capture ELISA for recombinant proteins fused to glutathione S-transferase: validation for HPV serology. *J Immunol Methods.* 2001;253(1-2):153-162.

92. Majewski S, Jablonska S. Skin autografts in epidermodysplasia verruciformis: human papillomavirus-associated cutaneous changes need over 20 years for malignant conversion. *Cancer Res.* 1997;57(19):4214-4216.

93. Bouwes Bavinck JN, Plasmeijer EI, Feltkamp MC. Beta-papillomavirus infection and skin cancer. *J Invest Dermatol.* 2008;128(6):1355-1358.

94. Jablonska S, Orth G. Epidermodysplasia verruciformis. *Clinics in dermatology.* 1985;3(4):83-96.

95. Harwood CA, Surentheran T, McGregor JM, et al. Human papillomavirus infection and non-melanoma skin cancer in immunosuppressed and immunocompetent individuals. *J Med Virol.* 2000;61(3):289-297.

96. Purdie KJ, Sexton CJ, Proby CM, et al. Malignant transformation of cutaneous lesions in renal allograft patients: a role for human papillomavirus. *Cancer Res.* 1993;53(21):5328-5333.

97. Casabonne D, Lally A, Mitchell L, et al. A case-control study of cutaneous squamous cell carcinoma among Caucasian organ transplant recipients: the role of antibodies against human papillomavirus and other risk factors. *Int J Cancer.* 2009;125(8):1935-1945.

98. Masini C, Fuchs PG, Gabrielli F, et al. Evidence for the association of human papillomavirus infection and cutaneous squamous cell carcinoma in immunocompetent individuals. *Archives of dermatology.* 2003;139(7):890-894.

99. Aldabagh B, Angeles JG, Cardones AR, Arron ST. Cutaneous squamous cell carcinoma and human papillomavirus: is there an association? *Dermatologic surgery : official publication for American Society for Dermatologic Surgery [et al.].* 2013;39(1 Pt 1):1-23.

100. Plasmeijer EI, Struijk L, Bouwes Bavinck JN, Feltkamp MC. Epidemiology of cutaneous human papillomavirus infections. *Cancer Treat Res.* 2009;146:143-157.

101. Waterboer T, Neale R, Michael KM, et al. Antibody responses to 26 skin human papillomavirus types in the Netherlands, Italy and Australia. *J Gen Virol.* 2009;90(Pt 8):1986-1998.

102. Accardi R, Gheit T. Cutaneous HPV and skin cancer. *Presse medicale (Paris, France : 1983).* 2014;43(12P2):e435-e443.

103. Mejlhede N, Pedersen BV, Frisch M, Fomsgaard A. Multiple human papilloma virus types in cervical infections: competition or synergy? *APMIS.* 2010;118(5):346-352.

104. Schiffman M, Clifford G, Buonaguro FM. Classification of weakly carcinogenic human papillomavirus types: addressing the limits of epidemiology at the borderline. *Infect Agent Cancer.* 2009;4:8.

105. Moscicki AB, Schiffman M, Burchell A, et al. Updating the natural history of human papillomavirus and anogenital cancers. *Vaccine.* 2012;30 Suppl 5:F24-33.

106. Kreimer AR, Clifford GM, Boyle P, Franceschi S. Human papillomavirus types in head and neck squamous cell carcinomas worldwide: a systematic review. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology.* 2005;14(2):467-475.

107. Zandberg DP, Bhargava R, Badin S, Cullen KJ. The role of human papillomavirus in nongenital cancers. *CA Cancer J Clin.* 2013;63(1):57-81.

108. Giuliano AR, Nyitray AG, Kreimer AR, et al. EUROGIN 2014 roadmap: differences in human papillomavirus infection natural history, transmission and human papillomavirus-related cancer incidence by gender and anatomic site of infection. *Int J Cancer.* 2015;136(12):2752-2760.

109. Mooij SH, van der Klis FR, van der Sande MA, et al. Seroepidemiology of high-risk HPV in HIV-negative and HIV-infected MSM: the H2M study. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology.* 2013;22(10):1698-1708.

110. Liu G, Markowitz LE, Hariri S, Panicker G, Unger ER. Seroprevalence of 9 Human Papillomavirus Types in the United States, 2005-2006. *The Journal of infectious diseases.* 2016;213(2):191-198.

111. Heiligenberg M, Michael KM, Kramer MA, et al. Seroprevalence and determinants of eight high-risk human papillomavirus types in homosexual men, heterosexual men, and women: a population-based study in Amsterdam. *Sex Transm Dis.* 2010;37(11):672-680.

112. Scherpenisse M, Mollers M, Schepp RM, et al. Seroprevalence of seven high-risk HPV types in The Netherlands. *Vaccine.* 2012;30(47):6686-6693.

113. Zhang C, Liu F, Deng Q, et al. Seroprevalence of 10 human papillomavirus types in the general rural population of Anyang, China: a cross-sectional study. *Sex Transm Infect.* 2015;91(7):506-509.

114. Newall AT, Brotherton JM, Quinn HE, et al. Population seroprevalence of human papillomavirus types 6, 11, 16, and 18 in men, women, and children in Australia. *Clin Infect Dis.* 2008;46(11):1647-1655.

115. Goldstone S, Palefsky JM, Giuliano AR, et al. Prevalence of and risk factors for human papillomavirus (HPV) infection among HIV-seronegative men who have sex with men. *The Journal of infectious diseases.* 2011;203(1):66-74.

116. Machalek DA, Poynten M, Jin F, et al. Anal human papillomavirus infection and associated neoplastic lesions in men who have sex with men: a systematic review and meta-analysis. *The Lancet. Oncology.* 2012;13(5):487-500.

117. Heiligenberg M, Alberts CJ, Waterboer T, et al. Route of sexual exposure is independently associated with seropositivity to HPV-16 and HPV-18 among clients of an STI clinic in the Netherlands. *The Journal of infectious diseases.* 2013;208(7):1081-1085.

118. Nyitray AG, Carvalho da Silva RJ, Baggio ML, et al. Six-month incidence, persistence, and factors associated with persistence of anal human papillomavirus in men: the HPV in men study. *The Journal of infectious diseases.* 2011;204(11):1711-1722.

119. Daling JR, Madeleine MM, Johnson LG, et al. Human papillomavirus, smoking, and sexual practices in the etiology of anal cancer. *Cancer.* 2004;101(2):270-280.

120. Vriend HJ, Bogaards JA, van der Klis FR, et al. Patterns of human papillomavirus DNA and antibody positivity in young males and females, suggesting a site-specific natural course of infection. *PLoS One.* 2013;8(4):e60696.

121. Lu B, Viscidi RP, Wu Y, et al. Seroprevalence of human papillomavirus (HPV) type 6 and 16 vary by anatomic site of HPV infection in men. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology.* 2012;21(9):1542-1546.

122. Syrjanen S, Waterboer T, Kero K, et al. Oral human papillomavirus infection in men might contribute to HPV serology. *European journal of clinical microbiology & infectious diseases : official publication of the European Society of Clinical Microbiology.* 2015;34(2):237-245.

123. Scherpenisse M, Mollers M, Schepp RM, et al. Changes in antibody seroprevalence of seven high-risk HPV types between nationwide surveillance studies from 1995-96 and 2006-07 in The Netherlands. *PLoS One.* 2012;7(11):e48807.

124. Masel J, Deiss RG, Wang X, et al. Seroprevalence and seroincidence of herpes simplex virus (2006-2010), syphilis (2006-2010), and vaccine-preventable human papillomavirus subtypes (2000-2010) among US military personnel. *Sex Transm Dis.* 2015;42(5):253-258.

APPENDICES

APPENDIX 1. LITERATURE REVIEW

A1.1. HUMAN PAPILLOMAVIRUS (HPV) VIRAL STRUCTURE AND PROTEINS

The HPV genome is a double-stranded circular DNA which is approximately 8 kb (7200-8000 base-pair) long (Figure 1).[1] HPV DNA can be divided into early (E), late (L) and long control (LCR, non-coding) regions.[1] These regions are separated by two (early and late) polyadenylation (pA) sites.[1] The early region constitutes more than 50% of the total viral genome and includes six open reading frames (ORFs) that encodes E1, E2, E4, E5, E6 and E7 viral proteins.[2] The late region, located downstream of the early region, forms 40% of the viral genome, and has L1 and L2 ORFs which encodes major (L1) and minor (L2) viral capsid proteins. LRC forms 10% of the viral genome, and it is a non-protein coding region which regulates the process of viral replication[2]

The E1 viral protein is required for DNA replication. It binds with the origin of replication located in the LCR region, functions as helicase, and prepares viral genome for replication[3] Also, the E1 protein recruits other replication proteins such as DNA polymerase from the host cell.[2] E2 viral protein assists E1 to bind with the origin of replication, but its primary function is regulation of the transcription process.[4] Also, E2 has a negative regulatory mechanism with E6 and E7 viral oncogenes.[5] Active E2 inhibits the expression of E6 and E7 and overexpresses p53 gene of cell which results in apoptosis. Whereas inactivated E2 overexpresses E6 and E7 oncoproteins resulting in cellular transformation and genetic destabilization.[5,6] E4 viral protein is mainly found in the cytoplasm of the host cells and is expressed in the late phases of the infection. It facilitates viral release to the environment.[7] The E4 may also play a role in the host cell cycle by arresting the G2 phase.[7] E6 and E7, viral oncoproteins, are of particular interest because of their role in the HPV related malignant transformation of human cells. The major role of E6 is degrading tumor suppressor protein, p53, in the host cells which in turn results in the DNA destabilization. The E6 protein also alters several other cellular pathways and disturbs the cell cycle and the cell maturation process.[8,9] The E7 oncoprotein degrades the tumor suppressor protein, retinoblastoma (pRb), of the host cells. Together E6 and E7 prevent cell death by inhibiting apoptosis, promoting cell-cycle progression, and immortalizing keratinocytes so that the virus can continue to replicate.[8,9] L1 and L2 are capsid proteins, both are about 500 amino acids long. HPV virons contain about 10 times more L1 protein than L2. This is why it is called a major capsid protein.[10]

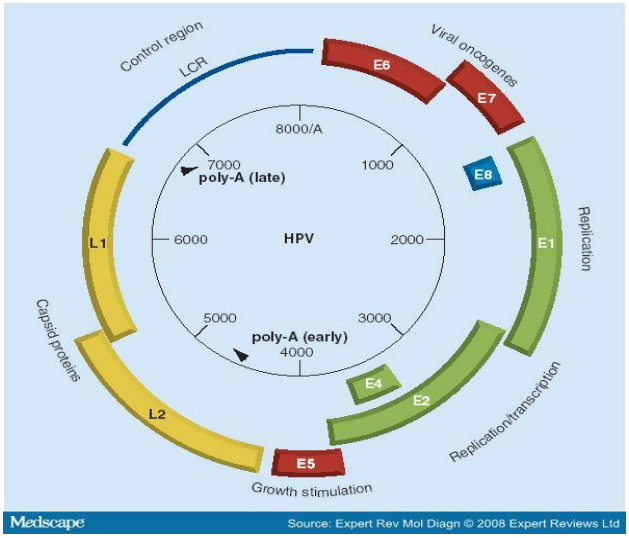

Figure A1.1. Human papillomavirus genome structure

This figure illustrates the genomic structure of HPV. The virus has three regions: Long control (LCR), early (E) and late (L) regions. LCR is a non-coding regulatory region. The E-region encodes E1, E2, E4, E5, E6 and E7 early proteins; and the L-region encodes L1 and L2 HPV capsid proteins.[6]

A1.2. CLASSIFICATION OF HPV

The Papillomaviridae family (also known papillomaviruses [PV]) includes highly diverse DNA viruses which infect mucosal and cutaneous epithelium of humans and other vertebrates.[11] PV are classified into 29 genera, which include over 200 types.[12] More than 120 types of PV are isolated from human cells hence called human papillomaviruses (HPV) and are classified into five genera: Alpha, Beta, Gamma, Mu, and Nu.[12,13] By convention, a new type of HPV is recognized if it is ≥10% different in the L1 open reading frame (ORF) region from the already sequenced types. About 60-70% of the nucleotides identity in the L1 region is shared by viruses that are placed in a particular genus.[14] L1 ORF is a highly conserved region of the HPV genome and from 2 to 10% variation in the nucleotides in the L1 region creates a sub-type, and variation in less than 1% creates a new variant.[12] Due to shortfalls in the classic, and immunological-based classifications of HPV, the genome-based approach is widely used.[11,12]

Bernard et al. has presented an expanded classification for PV using L1 nucleotide sequence, which contains 29 genera and 189 types. Out of these, 120 types are isolated from humans, 64 from the non-human mammals, 3 from birds and 2 from reptiles. Greek letters were used to designate genera (Figure A1.2).[11,12] The International Agency for Research on Cancer (IARC) expert group has classified mucosal HPV based on their carcinogenic potential into groups: 1A (high-risk), 2A (probable) and 2B (possible) cause for cancer. The high-risk group includes HPV 16, 18, 31, 33, 35, 45, 52, and 58, more constantly found in the cervical cancer; and HPV-39, 51, 56, and 59, less constantly found in the cervical cancer. Among the high-risk group, HPV 16 is the most important cause of cancer.[15] Due to limited evidence in humans and strong mechanistic evidence for cervical cancer, HPV 68 was classified as 2A; and due to limited evidence in humans, HPV 26, 30, 34, 53, 66, 67, 69, 70, 73, 82, 85, and 97 were classified as 2B or probable cause of cancer. HPV 6 and 11 were classified as group 3 (not carcinogens) due to inadequate epidemiological evidence and lack of carcinogenic mechanism. Beta HPV types 5 and 8 were classified as 2B (probable) and other gamma types were classified as group 3 (Table A1.1).[15]

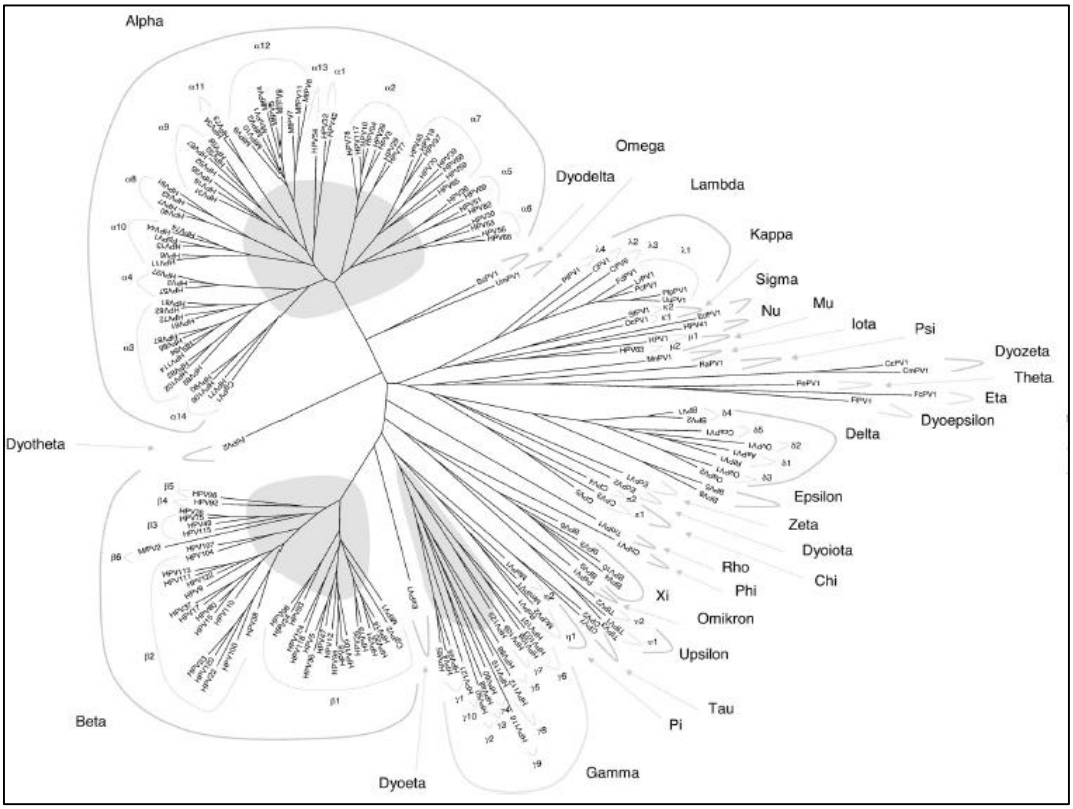

Figure A1.2. Phylogenetic tree of 189 papillomavirus types based on L1 nucleotide sequence*

*Greek letters represent genus and within each genus there are species ([groups] i.e. α1, α2, α3…α14), and the numbers at the end of each branch represent types. *Adopted from Bernard et al.*[12]

Table A1.1. The IARC classification of HPV*

Group	HPV types	Comments
Alpha HPV types		
1	16	Most potent HPV type, known to cause cancer at several sites
1	18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59	Sufficient evidence for cervical cancer
2A	68	Limited evidence in humans and strong mechanistic evidence for cervical cancer
2B	26, 53, 66, 67, 70, 73, 82	Limited evidence in humans for cervical cancer
2B	30, 34, 69, 85, 97	Classified by phylogenetic analogy to HPV types with sufficient or limited evidence in humans.
3	6, 11	
Beta HPV types		
2B	5 and 8	Limited evidence for skin cancer in patients with epidermodysplasia verrcuiformis
3	Other beta and gamma types	

**Adopted from A review of human carcinogens – Part B: biological agents* [15]

A.1.3. HPV LIFE CYCLE AND PATHOGENESIS

Unlike most viruses that produce their progeny from the infected cell directly, HPV relies on the mitosis and differentiation of the basal epithelial cells to produce its progeny. Understanding of the HPV lifecycle is mainly derived from research on HPV 16. However, with slight modifications, life cycle model of HPV 16 can be applied to other HPV types as well.[17] Skin to skin contact is the primary route of transmission, and sexual behavior greatly influences the incidence and prevalence of HPV infections.[18] Virus finds its way to basal epithelial cell via micro-abrasions and micro-traumas on the skin. It is not fully understood how the virus binds with the surface of basal cells. However, L1 and L2 capsid proteins, alpha-6 integrin cellular receptors, and heparan sulfate proteoglycans/heparin sulfate may play a role in the process of binding.[19] The virus enters basal epithelial cells through endocytosis, and the capsid is disassembled by the cellular endosome/lysosome. The L2 viral protein transfers the viral genome to nuclei of keratinocytes,[20] and with the help of early viral proteins (E1 and E2), the virus maintains its episomal form and replicates in synchrony with the host DNA.[17] As the infected host cells differentiate, late viral promoters are activated which facilitate viral packaging and release.[17,20] Usually the expression of L1 and L2 viral proteins takes place in the granular layer the viral assembly in the cornified layer of the skin.[7,20] Viruses are constantly released to the environment as the keratinocytes age and die.[17]

A.1.4. PREVALENCE OF CUTANEOUS HPV

Normal human skin hosts a diverse array of microbiota. Recent developments in meta-genomic techniques have revealed that a diverse array of viruses including cutaneous HPV, particularly beta and gamma genera represent a significant part of the skin flora.[21] Cutaneous HPV is also widely distributed in eyebrow hairs,[22] and external genital lesions (EGLs).[23] Until recently, about 51 β-HPV types and 80 γ -HPV types have been identified and sequenced.[24] Details of the types and sequence data can be found on (http://www.hpvcenter.se/html/refclones.html).

Hampras et al.[25] followed 209 men enrolled in the HIM study, a prospective study of the natural history of HPV infections in men, for a median of 12.6 months and measured DNA for 25 types of β cutaneous HPV and 16 types of γ cutaneous HPV in skin swabs and eyebrow hairs. They reported a prevalence of 67.3% for any β-HPV in the skin swabs, and 56.5% in the eyebrow hairs, and 15.9% for any γ-HPV in the skin swabs and 26.8% in the eyebrow hairs. In this study, the prevalence of 'any-HPV' was associated with older age in both types of specimens, (skin swabs, OR = 2.3; 95% CI: 1.2 – 4.6) and (eyebrow hairs, OR= 3.0; 95% CI: 1.2 – 7.0) for men over age 44 years compared with those age 18-33 years old , respectively.

One study[16] estimated seroprevalence of 36 cutaneous HPV among 411 patients recruited from a skin cancer screening clinic in Tampa Florida and reported a seroprevalence of 96% in men for ≥1 HPV types. Seroprevalence of γ-HPV type 4 was (46%), μ-HPV type 1 (37%) and β-HPV type 1 (31%). Another skin cancer case-control study[26] measured serum antibodies against major capsid L1 protein of cutaneous HPV type(s) within genera beta (β), gamma (γ), mu (μ) and nu (ν) among healthy controls and patient with squamous cell carcinoma (SCC). The study reported the following seroprevalence estimates for the control group: 33.3% for any β_1-HPV (types: 5, 8, 20, 24, 36); 47.7%

for any β_2-HPV (types: 9, 15, 17, 23, 38, 107); 33.7% for any β_3-HPV (types: 49, 75, 76); 16.7% for any β_4-HPV (type 92); and 15.0% for any β_5-HPV (type 96). Seroprevalence of γ-HPV type 4 was 34.3%, and the seroprevalence of μ-HPV type 1 and ν-HPV type 41 were 32.0% and 11.3% respectively. Another study[27] estimated seroprevalence of cutaneous HPV in a hospital-based case-control study. Cases were non-melanoma skin cancer patients and controls were patients with benign skin lesions. The study reported an overall seroprevalence of 42% for any β-HPV among the controls. Type-specific seroprevalence of antibodies against the major capsid L1 protein were reported as follows: β-HPV type 20 (14%), β-HPV type 5 (15.0%), β-HPV type 36 (7.0%), β-HPV type 8 (26.0%), β-HPV type 24 (11.0%), β-HPV type 15 (12.0%), β-HPV type 9 (12.0%), β-HPV type 38 (18.0%) and ν-HPV type 1 (18.0%).

One case series[23]of 69 men included pathologically confirmed EGL patients and reported DNA prevalence of 61.1% for 25 β-HPV (types: 5, 8, 12, 14, 19, 20, 21, 24, 25, 36, 47, 93, 9, 15, 17, 22, 23, 37, 38, 80, 49, 75, 76, 92 and 96) detected on the surfaces of EGLs with the most common types being 38 (16.7%), 5 (15.3%), and 12 (12.5%). Another study[24] estimated DNA prevalence of 43 β-HPV types and 30 γ-HPV types in anal samples obtained from 66 HIV-positive men sex with men (MSM), and 153 HIV-negative MSM. Among HIV-positive MSM, the study reported DNA prevalence of β-HPV (65.6%) and γ-HPV (68.2%), and among HIV negative MSM β-HPV (59.1%) and γ-HPV (57.7%) respectively. The study also reported a significantly higher DNA prevalence of β-HPV type 1, β-HPV type 3, γ-HPV type 6, and γ-HPV type 7 among HIV positive MSM compared to HIV negative MSM. One study assessed DNA prevalence of HPV types (5, 8, 9, 12, 14, 15, 17, 19-25, 36-38, 47 and 49) among 26 renal transplant patients and 22 healthy individuals in hairs plucked from eyebrows, scalp, arms and legs. The study reported a DNA prevalence of 92% for ≥1 HPV types among the renal transplant patients, and 45% among the healthy individuals.[28]

Very few studies have assessed the prevalence and persistence of cutaneous HPV among healthy individuals.[22,29] One study[22] assessed prevalence and persistence of 25 types β-HPV among 23 immuno-competent individuals over a period of two years and collected eight consecutive hair samples plucked from eyebrows. The study reported the baseline DNA prevalence to at least one β-HPV (61%) which increased to (96%) during the follow-ups with the most common type being β-HPV type 23. Type-specific β-HPV DNA was persistent for six months or longer in 74% cases.

Antonsson et al.[30] examined skin swab specimens obtained from newborns and infants, their mothers and environmental samples for cutaneous HPV DNA. On the day of birth, two of 16 babies, and on the second day of life, 8 of 16 babies, tested positive for cutaneous HPV DNA. They reported that cutaneous HPV DNA was detected in over 50% of the samples obtained from 1-month, 1-year and 4-year old children. Similarly, about 77% of the samples obtained from the mothers and 18% of the environmental samples (mother's bed-side table in the maternity ward) tested positive for cutaneous HPV DNA. The finding that HPV DNA is already present in the early life shows that it is environmentally ubiquitous. Another multinational study[31] assessed for cutaneous HPV DNA in the skin swabs obtained from women in five countries and reported the following prevalence estimates: Bangladesh 68%, Japan 54%, Ethiopia 52%, Zambia 42% and Sweden 70%. HPV 5 was the most commonly detected type.

In summary, the prevalences of cutaneous HPV differ across studies depending on whether the estimates are based on DNA detection or serum antibodies, whether the population is healthy or sick, older or younger. Table A1.2 shows a comparison for various cutaneous HPV types across four studies. It can be seen that estimates are also different based on the site of sample collection (e.g. eyebrow specimens or skin swabs) and the population being investigated. Different studies have included different sets of cutaneous HPV with the most common being beta types 5, 8, 9, 20 and 38. Also, statistical methods and reported measures varied across studies. Some studies reported prevalence estimates based on genus (e.g. β-HPV, γ-HPV), while other studies reported positivity to one type, two types, three types and so on. The prevalence estimates to at least one HPV ('any-HPV') increased when the number of the types in the study increased, whereas the prevalence estimates decreased when concurrent seropositivity was measured for more than one type (i.e., positive for 2 types or 3 types).

Table A1.2. DNA distribution and antibodies prevalence of various cutaneous HPV types

	Innacone et al.[16,a]	Iannacone at al.[26]		Andersson et al.[27]			Pierce-Campbell et al[23,b]		Hampras et al.[25]	
	Seroprevalence (%)	Seroprevalence (%)		Seroprevalence (%)			DNA Prevalence (%)		DNA Prevalence (%)	
HPV types	Screening Clinic Patients	Controls	SCC	SCC	BCC	Controls	Condyloma	PeIN	Eyebrow	Skin
HPV 27 (α)	18.0	11.0	16.8	-	-	-	-	-	-	-
HPV 5 (β)	14.0	12.7	20.2	19.0	13.0	13.0	21.0	0.0	7.7	17.3
HPV 8 (β)	31.0	23.0	36.0	29.0	23.0	26.0	17.9	0.0	6.7	4.5
HPV 9 (β)	24.0	25.0	29.5	21.0	8.0	12.0	7.1	16.7	5.7	6.4
HPV 12 (β)	-	-	-	-	-	-	10.7	0.0	9.6	19.9
HPV 14 (β)	-	-	-	-	-	-	17.9	0.0	2.4	3.8
HPV 17 (β)	25.0	25.7	35.3	-	-	-	10.7	16.7	6.2	10.3
HPV 20 (β)	21.0	21.0	29.5	13.0	13.0	13.0	0.0	0.0	4.8	3.2
HPV 22 (β)	-	-	-	-	-	-	14.3	0.0	8.1	6.4
HPV 23 (β)	19.0	-	-	-	-	-	14.3	0.0	13.4	8.3
HPV 24 (β)	20.0	12.3	20.8	17.0	9.0	11.0	7.1	0.0	-	-
HPV 38 (β)	20.0	24.7	24.3	25.0	16.0	18.0	21.4	16.7	15.3	14.1
HPV 47 (β)	-	-	-	-	-	-	10.7	0.0	3.3	5.8
HPV 4 (γ)	46.0	34.3	43.4	-	-	-	-	-	6.2	13.4
HPV 1 (μ)	37.0	32.0	38.2	21.0	19.0	18.0	-	-	-	-
HPV 41 (η)	14.0	11.3	12.1	-	-	-	-	-	-	-

a. Patients were recruited from a skin cancer screening clinic in Tampa Florida
b.A case series of 69 subjects, 28 condyloma and 6 PeIN cases.

A.1.5. CUTANEOUS HPV AND NON-MELANOMA SKIN CANCER

Emerging evidence suggests that cutaneous HPV is associated with the risk for non-melanoma skin cancer. Before this association is further described, a brief note is presented on skin cancer. Normal skin consists of three layers, the epidermis, dermis and the sub-cutaneous layer.[32] The epidermis is the top layer of the skin and consists of three main types of cells: squamous cells, which are constantly shed and replaced; basal cells, which constantly divide and replace the shed squamous cells; and melanocytes which give color to skin. The dermis is the middle layer of skin that contains hair follicles, sweat glands, blood vessels, nerves and collagen fibers. The sub-cutaneous layer is the deepest layer and is composed mainly of fat cells.[32,33] Skin cancer is the most common type of cancer among Caucasians worldwide. There are three main types of skin cancer: a) basal cell carcinomas (BCC), squamous cell carcinomas (SCC), and c) melanomas. The most common type of skin cancers are BCC and SCC, and together they are also called non-melanoma skin cancer (NMSC).[34] NMSC are mainly found on the sun-exposed parts of the body such as the head and neck. NMSCs are strongly related with sun exposure.[35,36] The other less common form of skin cancer is melanoma which originates from melanocytes.[37] Melanoma can be found anywhere on the body, most commonly on the chest and the back in men and on the legs in women.[37] Annually, around 5 million skin cancer patients are treated in the United States. In 2006, an estimated 3.5 million new cases of skin cancer were diagnosed in 2.2 million people.[38] The lifetime risk for skin cancer for Americans is one in five,[39,40] Around 2.8 million patients with BCC and another 0.7 million with SCC are diagnosed annually in the U.S.[40] In the past few decades, SCC incidence has doubled.[41] Close to 90% of NMSC and 85% of melanomas are associated with UV radiation from sun exposure.[42,43] In 2002, nearly 374,000 cases of NMSC were diagnosed and the age-standardized rate per 100 000 persons for NMSC in Australia was 1170 (BCC 884, SCC 387).[44]

Epidemiologic evidence examining the association of UV and skin cancer can be summarized as follows. Studies have reported a negative correlation between latitude of residence and incidence and mortality of NMSC and melanoma.[45] Animal models have shown that skin cancer could be produced by exposing mice to UV radiation.[46] Patients with genetic disorders who cannot repair UV-induced DNA damage adequately have higher incidence rates of skin cancers.[46] UV-carcinogenesis is a complex multistage process that involves cancer initiation, promotion and

progression. DNA damage is induced by UV radiation, which is promoted by UV-induced immunosuppression and other host and environmentally related factors to pre-cancerous and cancerous lesions.[47,48]

A number of studies have assessed the association between cutaneous HPV and skin cancer.[26,49-52] One study[26] examined the association between cutaneous HPV (genera: α, β, γ, μ and ν) and SCC comparing 173 SCC cases to 300 controls in a clinic-based case-control study conducted in Tampa, Florida. Serum antibodies against L1 capsid protein were tested for genera alpha (2, 3, 7, 10, 27, 57, and 77); beta (5, 8, 9, 15, 17, 20, 23, 24, 36, 38, 49, 75, 76, 92, 96, and 107); gamma (4, 48, 50, 65, 88, 95, 101, and 103); mu (1); and nu (41). The study found that serum antibodies against any HPV in genus-beta was positively associated with increased risk of SCC (OR = 1.93; 95% CI: 1.23–3.02). Type-specific associations for β-HPV type 8 was (OR= 1.80; 95% CI: 1.14–2.84), for β-HPV type 17 (OR=1.59; 95% CI: 1.02–2.49), and for α-HPV 10 (OR= 2.24; 95% CI: 1.04–4.85).

A multicenter case-control study,[49] compared 689 SCC patients with 845 controls recruited from the Netherlands, Italy and Australia. Serum antibodies against L1 capsid protein and DNA in eyebrow specimens were tested for 15 HPV types of genus beta (types: 5, 8, 9, 15, 17, 20, 23, 24, 36, 38, 49, 75, 76, 92, and 93). The study reported that any-beta HPV DNA was associated with an increased risk for SCC in the Netherlands (OR = 2.8; 95% CI: 1.3-5.8) and Italy (OR= 1.7; 95%CI: 0.79-3.6), but not in Australia (OR = 0.91; 95% CI: 0.53-1.6). In addition, serum antibodies against any β-HPV were positively associated with SCC in Australia (OR= 2.2; 95% CI: 1.4-3.3), the Netherlands (OR = 2.0; 95% CI: 1.2-3.4) and Italy (OR= 1.6, 95% CI: 0.94- 2.7). A stronger association between UV susceptibility and SCC among β-HPV seropositive individuals also was reported, supporting the possible interaction between cutaneous HPV and UV exposure

Another case-control study,[50] investigated the association between the presence of DNA of β-cutaneous HPV types 5, 8, 15, 20, 24 and 38 in eyebrow hairs and the risk for SCC in 155 immunocompetent SCC patients and 371 controls. The study reported age- and sex- adjusted odds ratios of 1.70 (95% CI: 1.10-2.70) for increased risk for SCC among people who tested positive to at least one of these types of HPV. Neale et al.[51] hypothesized that higher viral load of β-cutaneous HPV might be responsible for the increased risk of SCC. They conducted a study examining the association between eight β-cutaneous HPV (types: 5, 8, 15, 20, 23, 24, 36 and 38) viral load measured in eyebrow hair follicles and the risk of SCC in a hospital-based case-control study comparing 448 immunocompetent SCC cases with 464 controls from Italy and Australia, and 179 organ transplant recipients (OTR) SCC cases with 318 controls Compared with the lowest tertile, participants in the highest tertile of cumulative β-cutaneous HPV viral load were at a significantly higher risk for SCC in Australia and the in OTR group.

In a population-based case control study conducted in the U.S., Karagas et al.[52] compared 663 SCC and 898 BCC cases with 805 controls. Serum antibodies against L1 capsid protein were tested for 16 β-cutaneous HPV (types: 5, 8, 9, 15, 17, 20, 23, 24, 36, 38, 49, 75, 76, 92, 96 and 107). Seropositivity to β-cutaneous HPV was positively associated with SCC but not with BCC. Also, a dose-response association was reported where the odds ratios increased with increasing numbers of seropositivity to β-cutaneous types (P for trend = 0.003). Age, sex, education, smoking and skin sensitivity adjusted odds ratios were as follows: For one type β-cutaneous HPV positive: 0.99 (95% CI: 0.74–1.33); for 2-3 types positive: 1.44 (95% CI: 1.03 – 2.01); for 4-8 types positive 1.51 (95% CI: 1.03 – 2.20); and for more than 8 types positive 1.71 (95% CI: 1.12 – 2.62). These data suggest that unlike mucosal HPV types, cutaneous HPV might work in a group in the carcinogenesis of SCC.

In summary, the literature is consistent in showing that cutaneous HPV is positively associated with the risk for SCC of the skin. The association remains present after controlling for age and other NMSC risk factors, and is consistently reported for cutaneous HPV in genus beta, particularly for HPV 5, 8 and 10. Studies show that this association is more pronounced in ORT and immunocompromised patients who are at increased risk for both cutaneous HPV infections and SCC. Although the exact mechanism of this association is not fully understood, an interaction between UV exposure and cutaneous HPV infections might be responsible. It is also suggested that cutaneous HPV might work as group of viruses in carcinogenesis of SCC, unlike the mucosal HPV types where the majority of the cancers are caused by two types (HPV 16 and 18). However, the temporality of this association has not yet been established as the only prospective study of the association is on-going and has not yet reported data.

A.1.6. RELATIONSHIP OF HPV, PENILE CANCER, PeIN, AND GENITAL WARTS

In this section, we begin with a brief description of the anatomy and histology of the penis, penile cancer, its incidence prevalence and potential risk factors for penile cancer. Subsequently, a description of the distribution of mucosal HPV in penile cancer/penile intraepithelial neoplasia (PeIN) and genital warts will be presented. Finally, the section will be concluded with cutaneous HPV distribution in penile cancer/PeIN and genital warts.

The human penis is an intromittent organ of the male reproductive system which also serves as a urinal duct. The penis is made of three parts, root (radix), body (corpus) and glans. The root consists of the median urethral bulb and crura which attaches the penis to the fasciae and pubic rami in the perineum. The body includes three masses of erectile tissues, two on both sides are corpora cavernosa, and below them is corpus spongiosum through which the urethra passes. At birth, the glans is covered by foreskin or prepuce which is often removed in infants through circumcision.[53,54] The body and glans are covered by a thin stratified squamous epithelium, loosely connected to the deeper parts of the body of the penis, and is non-keratinized at glance before circumcision.[55] The skin of the penis is an extension of the lower abdominal-wall skin. It covers the body of the penis and joins the smooth and hairless skin of the glans penis at the corona. The subcutaneous tissues of the penile skin are rich in smooth muscles, also called Dartos Facial, which continues into the perineum and fuses with the perineal fasciae.[53,54]

Over 95% of penile cancers are of the squamous cell carcinoma (SCC) type.[56] SCC can develop anywhere on the penis, but the most common sites are the glans and foreskin. Generally, SCC of the penis grows slowly over many years. Before the development of a fully invasive penile cancer, pre-malignant changes occur in the skin cells of the penis.[56] Cancerous cells restricted to the penile skin that have not spread to any deeper tissues, are called carcinoma in situ or penile intraepithelial neoplasia (PeIN).[56] Penile cancer is relatively rare in developed countries comprising only 1% of all cancers in men.[57] The age-standardized incidence of penile cancer in Europe and the U.S. is about 1 per 100,000 men.[57] However, penile carcinoma is relatively common in developing countries comprising up to 10% of all malignancies in men,[58] and the incidence ranges from 3 per 100,000 men in India to 8.3 per 100,000 men in Brazil. Penile cancer incidence rates in Uganda are higher where the cumulative incidence approaches 1% by age 75.[58] Over 25% of penile cancer metastases are usually to the inguinal lymph nodes.[56]

Recent studies show that incidence rates of penile cancer are on the rise in some European countries.[59] The epidemiology of penile cancer is mainly drawn from case-reports and case-series, and very few population-based studies exist on this disease. In a population-based study, Hernandez et al.[60] used SEER and the 'National Program for Cancer Registries' data from 1998–2003 to estimate the incidence of invasive penile cancer in the U.S. During this time period, a total of 4967 cases of histologically confirmed invasive SCC of the penis were diagnosed. The annual incidence rate was estimated at 0.81 per 100,000 men. They also reported that the annual incidence increased steadily with age (RR = 8.03; 95% CI: 7.25 – 8.89, for 50-59 years vs. <50, and RR=18.48; 95% CI: 16.81-20.33, for 60-69 years vs. <50). Whites and African-Americans did not have significantly different incidence rates. Pacific Islanders had lower incidence rates (RR= 0.45; 95% CI: 0.35 – 0.58, vs. Whites), and Hispanics had higher incidence rates (RR=1.72; 95%CI: 1.56 – 1.88, vs. non-Hispanics). Similarly, the incidence rate was 16% higher in those counties where ≥ 25% of the population was living at the poverty level compared to those counties where ≤10% of the population was living at the poverty level. The incidence rates were also higher in areas where fewer men had completed high-school.[60]

The single most important and preventable cause of penile cancer is HPV. It is estimated that over 50% of the penile cancer is caused by HPV with the main types being HPV 16 (in 60% cases) and HPV 18 (in 15% cases).[57] HPV causes two types of genital lesions in men, condyloma (genital warts) and PeIN (penile intraepithelial neoplasia).[61] History of condyloma is associated with a 3-5 fold increased risk of penile cancer.[62] Dillner et al.[63] conducted a systematic review to describe the etiology of SCC of the penis and found that between 70 and 100% of PeIN cases were positive for HPV DNA, and over 50% of the invasive penile cancer tumors were positive for HPV DNA. The review also reported that more sensitive methods of HPV sequencing (i.e., PCR), compared to less sensitive methods (i.e., Southern Blot Hybridization assays), had higher rates of HPV detection in PeIN (5-48% vs. 17-82%). HPV 16 was the most frequently detected type.[63] Other risk factors for SCC of the penis are: i) phimosis (non-retractable foreskin);[64] ii) lack of circumcision, found associated in ecologic data; iii) smoking, consistently independently associated in a dose-response manner (3-fold risk for current vs. never smokers);[65,] iv) number of lifetime partners, with a 3-fold increased risk for ≥ 30 lifetime partners;[60] and v) history of condyloma, associated with a 3-5 fold increased risk.[63,65] Based on SCC histology, HPV prevalence is higher in purely basaloid forms (75%) of the penile cancer, and basaloid and/or warty forms (47%) as compared to the keratinizing form of penile cancer (11%).[63] Over 94% of the HPV-positive penile carcinomas are located on the glans or coronal sulcus.[60]

The role of circumcision in the prevention of penile cancer is somewhat controversial. Some studies suggest it is protective, others suggest that this association is confounded by other risk factors, such as sexual behavior. A systematic review[66] of eight studies assessed the association between male circumcision and penile cancer. Three studies in the review found a protective effect for childhood/adolescent circumcision (summary OR = 0.33; 95% CI: 0.13-0.83), two studies did not find any association after adjusting for phimosis, and the remaining three studies reported an increased risk of adult circumcision and SCC of the penis (summary OR=2.71; 95% CI: 0.93-7.94; 3

studies). The review concluded that circumcision is only protective if performed during childhood. Also, it is possible that circumcision corrects the problem of phimosis, which is an independent risk factor penile cancer.

Another systematic review[67] assessed HPV DNA distribution in invasive penile carcinoma. The review included 30 studies from different countries. A total of 1266 biopsy-confirmed penile carcinoma cases were included in the analysis. HPV DNA distribution was confirmed through PCR. The review reported that any-HPV prevalence in the penile SCC was (47.9%) with the highest prevalence in basaloid/warty histologic type (66.3%). Type-specific prevalence was (30.8%) for HPV 16, (6.7%) for HPV 6 and (6.6%) for HPV 18. In a retrospective cohort study of 216 men with invasive penile carcinoma diagnosed between 1984 and 2008 in Sweden, Kirrander et al.[68] estimated the overall and type-specific prevalence of HPV. Using real-time PCR, they detected HPV DNA in (82.9%) of tumor specimens. The majority of the tumors were infected with one type of HPV (70.4%), two types of HPV (25.7%) and three types of HPV (3.9%). Some studies have also assessed anti-HPV antibodies among penile cancer patients. Heideman et al.[69] compared 83 penile SCC patients with 83 age-matched controls and found that penile SCC patients were 6.7 times more likely to be seropositive for antibodies against L1 capsid protein for 'any HPV', and 11 times more likely to be seropositive for antibodies against E6 and E7 viral proteins for 'any HPV,' (OR = 6.7; 95% CI: 2.4 - 19) and (OR = 11; 95% CI: 3.6 – 34) respectively.

HPV is also associated with precursors of penile cancer, namely penile intraepithelial neoplasia(PeIN).[61,71] PeIN requires careful medical attention as from 1 to 30% of PeINs can progress to invasive penile cancer.[72] Based on the etiology, PeIN can be classified into HPV-related and HPV-unrelated.[19] Microscopically, PeIN is subdivided into differentiated and undifferentiated PeIN. The undifferentitated type is then sudivided into basaloid, warty, and warty-basaloid.[71] Figure A1.3 shows pre-malignant penile lesions. Between 70 and 100% of the PeIN are positive for HPV with the highest prevalence found among basaloid, warty, and wart-basaloid forms.[72] Very few studies have assessed the HPV distribution in PeIN. One study[73] estimated the prevalence of HPV DNA in genital lesions among men and compared two different methods, i) swabbing the surface of the lesions, and ii) tissue biopsies. It reported the following prevalence estimates for different types of HPV in 10 PeIN cases: Any HPV (100% both methods); HPV 6 (30% biopsy, 20% swab); HPV 11 (30% biopsy, 20% swab); HPV 16 (60% both methods); and HPV 18 (10% both methods). Another study[74] assessed HPV distribution in eight cases of Erythorplaisa of Queyart, a rare type of PeIN, and detected cutaneous HPV DNA in 100% of cases. HPV 16 DNA was detected in 88% of the cases, and DNA of HPV 39 and 51 were detected in 50% of the cases. One study[75] conducted in France used a Southern Blot method and reported the following HPV prevalence for different grades of PeIN. PeIN-I (43 cases): any HPV (83.7%), HPV 6 (33.3%), HPV 16 (38.9%) and HPV 18 (8.3%). PeIN-II (18 cases): any HPV (94.4%), HPV 6 (11.8%), HPV 16 (58.8%) and HPV 18 (11.8%). PeIN-III (4 cases): any HPV (100%) and HPV 16 (100%).

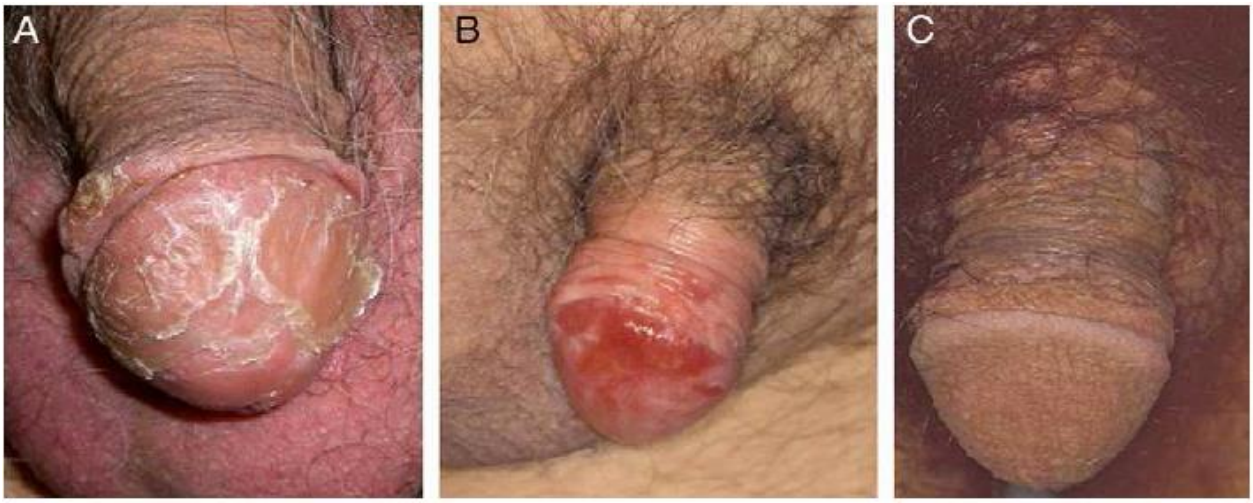

Figure A1.3. Pre-malignant or in situ lesions of the penis*

***A**, pseudoepitheliomatous micaceous balanitis. **B**, Erythroplasia of Queyrat. **C**, Bowenoid papulosis. *Adopted from Ferrandiz-Pulido et al.* [72]

Genital warts is a common STI in the U.S. and each year around one million new cases of genital warts are diagnosed.[76] Prevalence of genital warts decreases with increasing age and the highest prevalence among men is recorded at ages 25-29 years.[77] The ever prevalence of genital warts in the United States is estimated at 5.6% [78] Even though, genital warts are benign lesions, they cause considerable psychological discomfort to patients [79] The annual medical cost of genital warts in the U.S is estimated at $200 million [80] The duration between HPV exposure and the first clinical manifestation for a genital wart ranges between 3 weeks and 8 months[81], and about 20-30% of genital warts regress over time without any treatment,[82] however, the recurrence rate is also very high Sexual behavior is the most important risk factor for genital warts. Although genital warts are benign lesions mainly caused by HPV 6 and 11, men with a history of genital warts are 3-5 times more likely to develop SCC of the penis compared with men without a history of genital warts.[60,63,66] This association could be explained partially due to the common source of exposure (i.e., sexual contact) for both high-risk and low risk HPV. People with a history of genital warts often engage in sexual behaviors that increase their risk of acquiring high-risk HPV as well. In a large prospective cohort of men, Anic et al. [83] estimated the incidence of genital warts at 2.35 cases per 1,000 person-years, and the distribution of HPV DNA in genital warts was as follows: HPV 6 (43.8%), HPV 11 (10.7%) and HPV 16 (9.8%). They concluded that HPV 6 and 11 are the most common types of HPV detected in genital warts among men[83] In another study, Chang and colleagues [84] assessed the HPV DNA distribution in genital warts in China, and reported a prevalence of (78.1%) for low-risk HPV, (41.3%), for HPV 6 (37.6%) for HPV 11 and (10.4%) for HPV 16

In summary, some studies have assessed HPV DNA in invasive penile tumors. Few studies have examined HPV DNA distribution in PeIN and genital warts. In comparison to penile SCC, HPV DNA prevalence in PeIN is nearly doubled (70-100% vs. 50-60%). In part, this discrepancy can be explained by the notion that HPV-negative invasive penile cancers may arise from other cancer precursors. The majority of HPV prevalence estimates, both in invasive penile cancer and in PeIN, are drawn from case series and case reports. Case series are weak epidemiologic study designs and due to absence of a control group, they are inconclusive regarding the etiologic role of HPV in these lesions. Some studies were conducted 10-20 years ago. These studies may have provided underestimates of HPV prevalence in penile SCC because of the use of less sensitive DNA detection methods. Prospective data in this area are lacking. Prevalence of HPV DNA in PeIN/cancer varies in different populations and geographical areas. Some possible explanations for this variation could be that different populations have different sexual behaviors and other risk factors and that the use of different detection methods by different studies impede their comparison. Also, compared to PCR, Southern Blot tends to underestimate the prevalence. Very few sero-epidemiologic studies have assessed antibodies against HPV proteins in serum of PeIN or penile SCC patients. Although limited by the seroconversion rate and waning of antibodies over time, measurement of antibodies against L1 major capsid protein of HPV provides markers of cumulative exposure to HPV infection over time. Serologic studies could provide useful data on PeIN and penile cancer epidemiology There exists some limited literature assessing mucosal HPV distributions in penile SCC and PeIN. However, literature assessing the distribution of *cutaneous HPV* in penile cancer and/or PeIN is almost non-existent.

A1.7. HPV INFECTION IN MEN (*THE HIM STUDY*)

The HIM Study was a prospective study of the natural history of HPV infections in men which started in 2005 with over 4,000 participants recruited from South Florida, U.S., São Paulo, Brazil, and Cuernavaca, Mexico.[85, 86] Participants who met following criteria were recruited to the study: i) ages between 18 and 70 years, ii) residents of one of the three study sites, iii) did not have plans to relocate to another place in the following 4 years, iv) were willing to participate in the follow-up visits every 6 months for up to 4 years, v) no previous history of penile or anal cancers, vi) no previous history of HIV or AIDS , vii) no current history of discharges from penis or burning sensation during urination and were not receiving any treatment for any sexually transmitted infections, viii) no history of imprisonment or homelessness in the past 6 months, ix) no history of participation in the HPV vaccine studies. Study participants were recruited from the general population, universities, and organized healthcare systems in three age groups: 18–30 years, 31– 44 years and 45–70 years. In Brazil, the study was advertised through radio, television and newspapers, and participants were recruited from a STI clinic in Sao Paulo. In Mexico, members of a large health plan working in factories and military residing in Cuernavaca and Morelos were recruited. In the U.S., participants were recruited through brochures, flyers, posters, and advertisement in the local media from the University of South Florida and residence of the local communities in Greater Tampa Bay area. [85, 86]

REFERENCES

1. Zheng ZM, Baker CC. Papillomavirus genome structure, expression, and post-transcriptional regulation. *Frontiers in bioscience : a journal and virtual library.* 2006;11:2286-2302.
2. Stanley MA. Epithelial cell responses to infection with human papillomavirus. *Clinical microbiology reviews.* 2012;25(2):215-222.
3. Morshed K, Polz-Gruszka D, Szymanski M, Polz-Dacewicz M. Human Papillomavirus (HPV) - structure, epidemiology and pathogenesis. *Otolaryngologia polska. The Polish otolaryngology.* 2014;68(5):213-219.
4. Wilson VG, West M, Woytek K, Rangasamy D. Papillomavirus E1 proteins: form, function, and features. *Virus genes.* 2002;24(3):275-290.
5. Lin BY, Makhov AM, Griffith JD, Broker TR, Chow LT. Chaperone proteins abrogate inhibition of the human papillomavirus (HPV) E1 replicative helicase by the HPV E2 protein. *Molecular and cellular biology.* 2002;22(18):6592-6604.
6. McBride AA, McPhillips MG, Oliveira JG. Brd4: tethering, segregation and beyond. *Trends in microbiology.* 2004;12(12):527-529.
7. Longworth MS, Laimins LA. Pathogenesis of human papillomaviruses in differentiating epithelia. *Microbiology and molecular biology reviews : MMBR.* 2004;68(2):362-372.
8. Thomas JT, Hubert WG, Ruesch MN, Laimins LA. Human papillomavirus type 31 oncoproteins E6 and E7 are required for the maintenance of episomes during the viral life cycle in normal human keratinocytes. *Proceedings of the National Academy of Sciences of the United States of America.* 1999;96(15):8449-8454.
9. Oh ST, Longworth MS, Laimins LA. Roles of the E6 and E7 proteins in the life cycle of low-risk human papillomavirus type 11. *Journal of virology.* 2004;78(5):2620-2626.
10. Wang JW, Roden RB. L2, the minor capsid protein of papillomavirus. *Virology.* 2013;445(1):175-186.
11. De Villiers E-M, Fauquet C, Broker TR, Bernard H-U, zur Hausen H. Classification of papillomaviruses. *Virology.* 2004;324(1):17-27.
12. Bernard HU, Burk RD, Chen Z, van Doorslaer K, zur Hausen H, de Villiers EM. Classification of papillomaviruses (PVs) based on 189 PV types and proposal of taxonomic amendments. *Virology.* 2010;401(1):70-79.
13. Chouhy D, Bolatti EM, Perez GR, Giri AA. Analysis of the genetic diversity and phylogenetic relationships of putative human papillomavirus types. *The Journal of general virology.* 2013;94(Pt 11):2480-2488.
14. de Villiers EM. Cross-roads in the classification of papillomaviruses. *Virology.* 2013;445(1-2):2-10.
15. Bouvard V, Baan R, Straif K, et al. A review of human carcinogens—Part B: biological agents. *The lancet oncology.* 2009;10(4):321-322.
16. Iannacone MR, Michael KM, Giuliano AR, Waterboer T, Pawlita M, Rollison DE. Risk factors for cutaneous human papillomavirus seroreactivity among patients undergoing skin cancer screening in Florida. The Journal of infectious diseases. 2010;201(5):760-769.
17. Doorbar J. The papillomavirus life cycle. *Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology.* 2005;32 Suppl 1:S7-15.
18. Burd EM. Human papillomavirus and cervical cancer. *Clinical microbiology reviews.* 2003;16(1):1-17.
19. Letian T, Tianyu Z. Cellular receptor binding and entry of human papillomavirus. *Virology journal.* 2010;7:2.
20. Nguyen HP, Ramirez-Fort MK, Rady PL. The biology of human papillomaviruses. *Current problems in dermatology.* 2014;45:19-32.
21. Foulongne V, Sauvage V, Hebert C, et al. Human skin microbiota: high diversity of DNA viruses identified on the human skin by high throughput sequencing. *PloS one.* 2012;7(6):e38499.
22. de Koning MN, Struijk L, Bavinck JN, et al. Betapapillomaviruses frequently persist in the skin of healthy individuals. *The Journal of general virology.* 2007;88(Pt 5):1489-1495.
23. Pierce Campbell CM, Messina JL, Stoler MH, et al. Cutaneous human papillomavirus types detected on the surface of male external genital lesions: a case series within the HPV Infection in Men Study. *Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology.* 2013;58(4):652-659.
24. Torres M, Gheit T, McKay-Chopin S, et al. Prevalence of beta and gamma human papillomaviruses in the anal canal of men who have sex with men is influenced by HIV status. *Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology.* 2015;67:47-51.
25. Hampras SS, Giuliano AR, Lin HY, et al. Natural history of cutaneous human papillomavirus (HPV) infection in men: the HIM study. *PloS one.* 2014;9(9):e104843.

26. Iannacone MR, Gheit T, Waterboer T, et al. Case-control study of cutaneous human papillomaviruses in squamous cell carcinoma of the skin. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology.* 2012;21(8):1303-1313.
27. Andersson K, Waterboer T, Kirnbauer R, et al. Seroreactivity to cutaneous human papillomaviruses among patients with nonmelanoma skin cancer or benign skin lesions. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology.* 2008;17(1):189-195.
28. Boxman IL, Berkhout RJ, Mulder LH, et al. Detection of human papillomavirus DNA in plucked hairs from renal transplant recipients and healthy volunteers. *J Invest Dermatol.* 1997;108(5):712-715.
29. Hsu JY, Chen AC, Keleher A, McMillan NA, Antonsson A. Shared and persistent asymptomatic cutaneous human papillomavirus infections in healthy skin. *Journal of medical virology.* 2009;81(8):1444-1449.Y
30. Antonsson A, Karanfilovska S, Lindqvist PG, Hansson BG. General acquisition of human papillomavirus infections of skin occurs in early infancy. *Journal of clinical microbiology.* 2003;41(6):2509-2514.
31. Antonsson A, Erfurt C, Hazard K, et al. Prevalence and type spectrum of human papillomaviruses in healthy skin samples collected in three continents. *The Journal of general virology.* 2003;84(Pt 7):1881-1886.
32. Kanitakis J. Anatomy, histology and immunohistochemistry of normal human skin. *European journal of dermatology : EJD.* 2002;12(4):390-399; quiz 400-391.
33. McLafferty E, Hendry C, Alistair F. The integumentary system: anatomy, physiology and function of skin. *Nursing standard.* 2012;27(3):35-42.
34. Gordon R. Skin cancer: an overview of epidemiology and risk factors. *Seminars in oncology nursing.* 2013;29(3):160-169.
35. Mancebo SE, Wang SQ. Skin cancer: role of ultraviolet radiation in carcinogenesis. *Reviews on environmental health.* 2014;29(3):265-273.
36. Narayanan DL, Saladi RN, Fox JL. Review: Ultraviolet radiation and skin cancer. *International journal of dermatology.* 2010;49(9):978-986.
37. Marks R. Epidemiology of melanoma. *Clinical and Experimental Dermatology.* 2000;25(6):459-463.
38. American Cancer Society. Cancer Facts & Figures 2015. Available at http://www.cancer.org/acs/groups/content/@editorial/documents/document/acspc-044552.pdf. .
39. Robinson JK. Sun exposure, sun protection, and vitamin D. *Jama.* 2005;294(12):1541-1543.
40. Portal E. Skin Cancer Facts 2013.
41. Karia PS, Han J, Schmults CD. Cutaneous squamous cell carcinoma: estimated incidence of disease, nodal metastasis, and deaths from disease in the United States, 2012. *Journal of the American Academy of Dermatology.* 2013;68(6):957-966.
42. Parkin DM, Mesher D, Sasieni P. 13. Cancers attributable to solar (ultraviolet) radiation exposure in the UK in 2010. *British journal of cancer.* 2011;105 Suppl 2:S66-69.
43. Koh HK, Geller AC, Miller DR, Grossbart TA, Lew RA. Prevention and early detection strategies for melanoma and skin cancer. Current status. *Archives of dermatology.* 1996;132(4):436-443.
44. Staples MP, Elwood M, Burton RC, Williams JL, Marks R, Giles GG. Non-melanoma skin cancer in Australia: the 2002 national survey and trends since 1985. *The Medical journal of Australia.* 2006;184(1):6-10.
45. Elwood JM, Lee JA, Walter SD, Mo T, Green AE. Relationship of melanoma and other skin cancer mortality to latitude and ultraviolet radiation in the United States and Canada. *International journal of epidemiology.* 1974;3(4):325-332.
46. Armstrong BK, Kricker A. The epidemiology of UV induced skin cancer. *Journal of photochemistry and photobiology. B, Biology.* 2001;63(1-3):8-18.
47. Nishisgori C. Current concept of photocarcinogenesis. *Photochemical & photobiological sciences : Official journal of the European Photochemistry Association and the European Society for Photobiology.* 2015.
48. Cleaver JE, Crowley E. UV damage, DNA repair and skin carcinogenesis. *Frontiers in bioscience: a journal and virtual library.* 2002;7:d1024-1043.
49. Bouwes Bavinck JN, Neale RE, Abeni D, et al. Multicenter study of the association between betapapillomavirus infection and cutaneous squamous cell carcinoma. *Cancer research.* 2010;70(23):9777-9786.

50. Struijk L, Bouwes Bavinck JN, Wanningen P, et al. Presence of human papillomavirus DNA in plucked eyebrow hairs is associated with a history of cutaneous squamous cell carcinoma. *J Invest Dermatol.* 2003;121(6):1531-1535.
51. Neale RE, Weissenborn S, Abeni D, et al. Human papillomavirus load in eyebrow hair follicles and risk of cutaneous squamous cell carcinoma. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology.* 2013;22(4):719-727.
52. Karagas MR, Waterboer T, Li Z, et al. Genus beta human papillomaviruses and incidence of basal cell and squamous cell carcinomas of skin: population based case-control study. *Bmj.* 2010;341:c2986.
53. Society AC. Penile cancer. American Cancer Socity. Atlanta, GA. 2015.
54. Cubilla A, Chaux A. Penis and scrotum: Anatomy of penis. PathologyOutlines. 2013.
55. Taylor JR, Lockwood AP, Taylor AJ. The prepuce: specialized mucosa of the penis and its loss to circumcision. *British journal of urology.* 1996;77(2):291-295.
56. Yaghoobi Yeganeh Manesh J, Shafiee R, Mohammad Bahrami A, et al. Cyto-histopathological and outcome features of the prepuce squamous cell carcinoma of a mixed breed dog. *Diagnostic pathology.* 2014;9:110.
57. Arya M, Kalsi J, Kelly J, Muneer A. Malignant and premalignant lesions of the penis. *Bmj.* 2013;346:f1149.
58. Misra S, Chaturvedi A, Misra NC. Penile carcinoma: a challenge for the developing world. *The Lancet. Oncology.* 2004;5(4):240-247.
59. Baldur-Felskov B, Hannibal CG, Munk C, Kjaer SK. Increased incidence of penile cancer and high-grade penile intraepithelial neoplasia in Denmark 1978-2008: a nationwide population-based study. *Cancer causes & control : CCC.* 2012;23(2):273-280.
60. Hernandez BY, Barnholtz-Sloan J, German RR, et al. Burden of invasive squamous cell carcinoma of the penis in the United States, 1998-2003. *Cancer.* 2008;113(10 Suppl):2883-2891.
61. Sudenga SL, Ingles DJ, Pierce Campbell CM, et al. Genital Human Papillomavirus Infection Progression to External Genital Lesions: The HIM Study. *European urology.* 2015.
62. Doorbar J, Quint W, Banks L, et al. The biology and life-cycle of human papillomaviruses. *Vaccine.* 2012;30 Suppl 5:F55-70.
63. Dillner J, von Krogh G, Horenblas S, Meijer CJ. Etiology of squamous cell carcinoma of the penis. *Scandinavian journal of urology and nephrology. Supplementum.* 2000(205):189-193.
64. Tsen HF, Morgenstern H, Mack T, Peters RK. Risk factors for penile cancer: results of a population-based case-control study in Los Angeles County (United States). *Cancer causes & control : CCC.* 2001;12(3):267-277.
65. Denniston GC. Re: History of circumcision, medical conditions, and sexual activity and risk of penile cancer. *J Natl Cancer Inst.* 1993;85(15):1251.
66. Larke NL, Thomas SL, dos Santos Silva I, Weiss HA. Male circumcision and penile cancer: a systematic review and meta-analysis. *Cancer causes & control : CCC.* 2011;22(8):1097-1110.
67. Backes DM, Kurman RJ, Pimenta JM, Smith JS. Systematic review of human papillomavirus prevalence in invasive penile cancer. Cancer Causes & Control. 2009;20(4):449-457.
68. Kirrander P, Kolaric A, Helenius G, et al. Human papillomavirus prevalence, distribution and correlation to histopathological parameters in a large Swedish cohort of men with penile carcinoma. *BJU international.* 2011;108(3):355-359.
69. Heideman DA, Waterboer T, Pawlita M, et al. Human papillomavirus-16 is the predominant type etiologically involved in penile squamous cell carcinoma. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology.* 2007;25(29):4550-4556.
70. Lohneis P, Boral S, Kaufmann AM, et al. Human papilloma virus status of penile squamous cell carcinoma is associated with differences in tumour-infiltrating T lymphocytes. *Virchows Arch.* 2015;466(3):323-331.
71. Velazquez EF, Chaux A, Cubilla AL. Histologic classification of penile intraepithelial neoplasia. *Semin Diagn Pathol.* 2012;29(2):96-102.
72. Ferrandiz-Pulido C, de Torres I, Garcia-Patos V. [Penile squamous cell carcinoma]. *Actas Dermosifiliogr.* 2012;103(6):478-487.
73. Anic GM, Messina JL, Stoler MH, et al. Concordance of human papillomavirus types detected on the surface and in the tissue of genital lesions in men. *Journal of medical virology.* 2013;85(9):1561-1566.
74. Wieland U, Jurk S, Weissenborn S, Krieg T, Pfister H, Ritzkowsky A. Erythroplasia of queyrat: coinfection with cutaneous carcinogenic human papillomavirus type 8 and genital papillomaviruses in a carcinoma in situ. *J Invest Dermatol.* 2000;115(3):396-401.

75. IARC. Human Papillomavirus. IARC working group on the evaluation of carcinogenic risks to humans. Vol. 90. Lyon, France: International Agency for Research on Cancer, 2007 IARC monographs on the evaluation of carcinogenic risks).
76. Monk BJ, Tewari KS. The spectrum and clinical sequelae of human papillomavirus infection. Gynecol Oncol 2007;107:S6-13.
77. 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.
78. 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.
79. Jeynes C, Chung MC and Challenor R. 'Shame on you' - the psychosocial impact of genital warts. Int J STD AIDS 2009;20:557-60.
80. 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.
81. Lacey CJ. Therapy for genital human papillomavirus-related disease. J Clin Virol 2005;32 Suppl 1:S82-90.
82. Wiley DJ, Douglas J, Beutner K, et al. External genital warts: diagnosis, treatment, and prevention. Clin Infect Dis 2002;35:S210-24.
83. Anic GM, Lee JH, Stockwell H, et al. Incidence and human papillomavirus (HPV) type distribution of genital warts in a multinational cohort of men: the HPV in men study. *The Journal of infectious diseases.* 2011;204(12):1886-1892.
84. Chang L, Ci P, Shi J, et al. Distribution of genital wart human papillomavirus genotypes in China: a multi-center study. *Journal of medical virology.* 2013;85(10):1765-1774.
85. 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 epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology.* 2008;17(8):2036-2043.
86. Giuliano AR, Lee JH, Fulp W, et al. Incidence and clearance of genital human papillomavirus infection in men (HIM): a cohort study. *Lancet.* 2011;377(9769):932-940.

APPENDIX 2. SUPPLEMENT TABLES AND FIGURES

Table A2.1. Baseline characteristics of randomly selected men compared to the entire HIM cohort

	Full cohort (n=4290)		Sub-cohort (n=600)		
Characteristic	**n**	**%**	**n**	**%**	**p-value[a]**
Country					
U.S.	1426	33.24	186	31.00	0.404
Brazil	1442	33.61	200	33.33	
Mexico	1422	33.15	214	35.67	
Age, years					
18-30	2040	47.5	260	43.3	0.104
31-44	1646	38.3	256	42.7	
>45	613	14.3	84	14.0	
Race					
White	1886	44.72	271	45.70	0.529
Black	669	15.86	78	13.15	
Asian	119	2.82	17	2.87	
Native Hawaiian or Other Pacific Islander	3	0.07	0	0.0	
American Indian, Alaska Native	81	1.92	9	1.52	
Other	1459	34.60	218	36.76	
Ethnicity					
Hispanic	1926	47.15	282	47.88	0.740
Non-Hispanic	2159	52.85	307	52.12	
Education, years					
≤12	2105	49.27	287	48.07	0.339
13-15	1207	28.25	160	26.80	
≥16	960	22.47	150	25.13	
Marital Status					
Single, never married	1934	45.24	260	43.55	0.886
Married	1454	34.01	212	35.51	
Cohabiting, living together	511	11.95	67	11.22	
Divorced/separated	362	8.47	56	9.38	
Widowed	14	0.33	2	0.34	
Smoking Status					
Current	1038	24.20	147	24.50	0.984
Former	806	18.79	113	18.83	
Never	2446	57.02	340	56.67	
Circumcision					
No	2687	63.75	389	64.83	0.605
Yes	1528	36.25	211	35.17	
Sexual Orientation					
MSW	3565	88.11	526	88.85	0.800
MSM	121	8.90	51	8.61	
MSWM	360	2.99	15	2.53	
Alcohol Consumption, # drinks/month*					
Mean (SD)	33.94	(65.15)	36.41	(69.47)	0.694
Median (range)	9.00	(0-480)	8.00	(0-468)	
0	1024	24.17	141	23.90	0.790
1–30	1939	45.77	281	47.63	
31-60	463	10.93	58	9.83	
≥61	810	19.12	110	18.64	
Number of Female Sex Lifetime Partners					
Mean (SD)	17.06	(45.46)	19.61	(50.31)	0.417
Median (range)	8.00	(0-1000)	8.00	(0-700)	
0	83	2.05	60	10.71	**<0.001**
1-3	967	23.84	126	22.50	
4-18	1779	43.86	247	44.11	
≥19	1227	30.25	127	22.68	

(Continued on next page)

Table A2.1. (Continued)

Characteristics	Full cohort (n=4290) n	Full cohort (n=4290) %	Sub-cohort (n=600) n	Sub-cohort (n=600) %	p-value[a]
Number of Male Sex Lifetime Partners					
Mean (SD)	3.54	(44.31)	4.00	(42.46)	0.6450
Median (Range)	0.00	(0-2000)	0.00	(0-750)	
0	3309	85.59	508	85.38	0.5630
1	155	4.01	29	4.87	
≥2	402	10.40	58	9.75	

a. For categorical variables Chi-square and for continuous variables Wilcoxon rank-sum test were used to calculate p-values.

Significant p-value is marked in bold

Table A2.2. Type-specific cutaneous HPV seroprevalence among men residing in the U.S., Brazil, and Mexico[a,b]

Type of HPV	Overall Seroprevalence (N=598) n (%)	Overall Seroprevalence (N=598) 95% CI	U.S. Seroprevalence (N=184) n (%)	U.S. Seroprevalence (N=184) 95% CI	Brazil Seroprevalence (N=200) n (%)	Brazil Seroprevalence (N=200) 95%CI	Mexico Seroprevalence (N=214) n (%)	Mexico Seroprevalence (N=214) 95% CI
Any HPV	391(65.4)	[61.6-69.2]	121(65.8)	[58.8-72.7]	140(70.0)	[63.6-76.4]	130(60.7)	[54.2-67.3]
Any β	233(39.0)	[35.0-42.9]	72(39.1)	[32.0-46.2]	97(48.5)	[41.5-55.5]	64(29.9)	[23.7-36.1]
β-HPV 5	46(7.7)	[5.6-9.8]	12(6.5)	[2.9-10.1]	30(15.0)	[10.0-20.0]	4 (1.9)	[0.0-3.7]
β-HPV 8	122(20.4)	[17.2-23.6]	39(21.2)	[15.2-27.2]	59(29.5)	[23.1-35.9]	24(11.2)	[7.0-15.5]
β-HPV 12	36(6.0)	[4.1-7.9]	6(3.3)	[0.7-5.9]	26(13.0)	[8.3-17.7]	4(1.9)	[0.0-3.7]
β-HPV 14	34 (5.7)	[3.8-7.5]	7(3.8)	[1.0- 6.6]	22(11.0)	[6.6-15.4]	5(2.3)	[0.3-4.4]
β-HPV 17	78(13.0)	[10.3-15.8]	20(10.9)	[6.3-15.4]	39(19.5)	[14.0-25.0]	19(8.9)	[5.0-12.7]
β-HPV 22	41(6.9)	[4.8-8.9]	12 (6.5)	[2.9-10.1]	22(11.0)	[6.6-15.4]	7(3.3)	[0.9-5.7]
β-HPV 23	73(12.2)	[9.6-14.8]	22(12.0)	[7.2-16.7]	39(19.5)	[14.0-25.0]	12(5.6)	[2.5-8.7]
β-HPV 24	46(7.7)	[5.6-9.8]	13(7.1)	[3.3-10.8]	25(12.5)	[7.9-17.1]	8(3.7)	[1.2-6.3]
β-HPV 38	83(13.9)	[11.1-16.7]	26(14.1)	[9.1-19.2]	42(21.0)	[15.3-26.7]	15(7.0)	[3.6-10.5]
β-HPV 47	74(12.4)	[9.7-15.0]	24(13.0)	[8.1-18.0]	33(16.5)	[11.3-21.7]	17(7.9)	[4.3-11.6]
α-HPV 27	53(8.9)	[6.6-11.1]	17(9.2)	[5.0-13.5]	23(11.5)	[7.0-16.0]	13(6.1)	[2.8-9.3]
γ-HPV 4	185(30.9)	[27.2-34.7]	61(33.2)	[26.3-40.0]	75(37.5)	[30.7-44.3]	49(22.9)	[17.2-28.6]
μ-HPV 1	171(28.6)	[25.0-32.2]	69(37.5)	[30.4-44.6]	69(34.5)	[27.9-41.1]	33(15.4)	[10.5-20.3]
ν-HPV 41	56(9.4)	[7.0-11.7]	14(7.6)	[3.7-11.5]	24(12.0)	[7.5-16.5]	18(8.4)	[4.7-12.2]

a. 600 subjects were randomly selected at baseline from HIM study to participant in the cutaneous HPV seroprevalence study. Seroprevalence data for 2 subjects were not available therefore they were excluded from analysis.

b. Confidence limits were calculated using Wald (linear) confidence limits method.

Table A2.3. Factors independently associated with α, β, ν cutaneous HPV seroprevalence among men residing in the U.S., Brazil, and Mexico[a]

	α-HPV 27		ν-HPV 41		β-HPV 5		β-HPV 12		β-HPV 14	
Characteristics	**OR**	**AOR (95%CI)**	**OR**	**AOR (95%CI)**	**OR**	**AOR (95%CI)**	**OR**	**AOR (95%CI)**	**OR**	**AOR (95%CI)**
Country										
U.S.	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Brazil	1.28	1.54 (0.57 - 4.12)	1.66	1.02 (0.37 - 2.81)	**2.53**	**4.14 (1.49 -11.49)**	**4.43**	**10.0 (2.86 - 34.93)**	**3.13**	**7.14 (2.18 - 23.4)**
Mexico	0.64	0.46 (0.10 - 2.07)	1.12	0.53 (0.14 - 2.06)	**0.27**	0.35 (0.04 - 2.94)	0.57	0.21 (0.03 - 1.58)	0.60	0.40 (0.04 - 4.23)
Age, Years										
18-30	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
31-44	1.29	1.27 (0.62 - 2.62)	1.06	0.89 (0.45 - 1.75)	1.68	2.09 (0.91 - 4.79)	**2.66**	2.64 (0.99 - 7.05)	**2.37**	**3.83 (1.32 - 11.11)**
45-73	1.27	1.02 (0.37 - 2.83)	1.25	0.96 (0.39 - 2.41)	1.50	1.52 (0.49 - 4.72)	**3.35**	2.72 (0.82 - 9.10)	**3.35**	**5.59 (1.63 - 19.18)**
Race										
White	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Black	0.40	0.43 (0.14 - 1.31)	0.33	0.38 (0.11 - 1.32)	1.00	0.77 (0.31 - 1.96)	0.53	0.56 (0.17 - 1.84)	1.04	0.93 (0.31 - 2.83)
Asian/Am. Indian/ Alaska Native	0.62	0.76 (0.16 - 3.61)	1.50	0.84 (0.18 - 3.94)	0.31	0 (NE)	0.39	0.00 (NE)	1.04	1.60 (0.31 - 8.33)
Other	0.55	1.37 (0.34 - 5.60)	0.84	1.18 (0.33 - 4.20)	**0.18**	1.17 (0.17 - 7.89)	**0.28**	5.19 (0.89 - 30.43)	**0.35**	2.60 (0.30 - 22.69)
Education										
≤12	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
13-15	0.94	0.80 (0.34 - 1.93)	1.11	1.02 (0.45 - 2.32)	0.99	1.09 (0.44 - 2.70)	1.27	1.78 (0.64 - 5.00)	2.23	3.86 (1.39 - 10.67)
≥16	**2.08**	1.87 (0.91 - 3.86)	1.68	1.80 (0.89 - 3.63)	1.49	1.48 (0.64 - 3.41)	**2.19**	2.08 (0.82 - 5.25)	1.81	1.93 (0.68 - 5.50)
Circumcision										
No	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Yes	1.25	1.30 (0.56 - 3.01)	0.59	0.56 (0.23 - 1.34)	1.63	**2.66 (1.14 - 6.18)**	1.20	2.40 (0.98 - 5.88)	1.32	**2.63 (1.01 - 6.88)**
No. of Female LTP										
0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
1-3	0.65	0.78 (0.29 - 2.09)	0.61	0.61 (0.19 - 1.93)	0.85	1.20 (0.35 - 4.16)	0.79	1.21 (0.24 - 6.07)	1.46	2.06 (0.47 - 9.07)
4-18	0.39	0.41 (0.16 - 1.08)	1.20	1.17 (0.43 - 3.18)	0.87	0.80 (0.25 - 2.53)	1.41	1.36 (0.33 - 5.66)	1.23	1.13 (0.28 - 4.64)
≥19	0.60	0.47 (0.16 - 1.34)	0.69	0.59 (0.18 - 1.93)	1.05	0.50 (0.14 - 1.75)	1.46	0.70 (0.15 - 3.24)	0.79	0.41 (0.08 - 2.04)
No. of Male Anal LTP										
0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
1	0.44	0.46 (0.06 - 3.72)	0.79	0.77 (0.17 - 3.55)	1.78	1.47 (0.36 - 5.94)	0.71	0.45 (0.05 - 3.76)	0.00	0.00 (NE)
≥2	**3.13**	1.87 (0.80 - 4.35)	1.64	1.30 (0.51 - 3.31)	**3.09**	1.43 (0.57 - 3.55)	**3.54**	1.45 (0.54 - 3.84)	**3.54**	1.65 (0.60 - 4.56)

Note: OR= unadjusted OR; AOR = adjusted odds ratio; CI = confidence interval; Am.=American LTP = lifetime sex partners; significant results are marked in bold

a. Final model was selected through backward stepwise elimination with significance level of p≤0.1 for retention in the model. Country, age, race, marital status, education, smoking alcohol consumption, circumcision, # of female LTP, and # of male LTP were loaded to the model while forcing country and age (design factors) to stay in the model

b. 'Any-HPV' category included men seropositive for at least one HPV genotype, and 'Any β HPV' category included men seropositive for at least one β HPV types.

Table A2.3. (Continued)

	β-HPV 22		β-HPV 24	
Characteristics	**OR**	**AOR (95%CI)**	**OR**	**AOR (95%CI)**
Country				
U.S.	1.00	1.00	1.00	1.00
Brazil	1.77	1.49 (0.50 - 4.45)	1.88	**3.96 (1.44 - 10.89)**
Mexico	0.48	0.11 (0.02 - 0.58)	0.51	0.87 (0.12 - 6.14)
Age, Years				
18-30	1.00	1.00	1.00	1.00
31-44	**2.34**	**2.56 (1.06 - 6.20)**	1.61	1.46 (0.66 - 3.21)
45-73	**3.03**	**3.36 (1.16 - 9.75)**	1.74	1.47 (0.52 - 4.15)
Race				
White	1.00	1.00	1.00	1.00
Black	1.28	1.16 (0.45 - 3.04)	1.95	1.87 (0.79 - 4.41)
Asian/American Indian/Alaska Native	0.45	0.65 (0.08 - 5.57)	0.89	1.58 (0.33 - 7.65)
Other	0.54	3.67 (0.82 - 16.43)	0.46	1.61 (0.26 - 10.17)
Education				
≤12	1.00	1.00	1.00	1.00
13-15	1.02	1.10 (0.42 - 2.85)	1.38	1.55 (0.63 - 3.84)
≥16	2.04	1.72 (0.74 - 3.96)	**2.33**	**2.43 (1.08 - 5.48)**
Circumcision				
No	1.00	1.00	1.00	1.00
Yes	0.86	0.77 (0.30 - 1.97)	1.79	**2.43 (1.09 - 5.42)**
No. of Female LTP				
0	1.00	1.00	1.00	1.00
1-3	0.61	0.99 (0.30 - 3.31)	0.45	0.59 (0.18 - 1.94)
4-18	0.63	0.79 (0.26 - 2.39)	0.67	0.73 (0.26 - 2.04)
≥19	0.78	0.52 (0.15 - 1.76)	0.65	0.46 (0.15 - 1.46)
No. of Male Anal LTP				
0	1.00	1.00	1.00	1.00
1	0.00	0.00 NE)	1.18	1.22 (0.25 - 5.90)
≥2	**4.46**	2.32 (0.96 - 5.57)	**4.44**	**2.55 (1.07 - 6.05)**

Note: OR= unadjusted OR; AOR = adjusted odds ratio; CI = confidence interval; LTP = lifetime sex partners; significant results are marked in bold

a. Final model was selected through backward stepwise elimination with significance level of p≤0.1 for retention in the model. Country, age, race, marital status, education, smoking alcohol consumption, circumcision, # of female LTP, and # of male LTP were loaded to the model while forcing country and age (design factors) to stay in the model

b. 'Any-HPV' category included men seropositive for at least one HPV genotype, and 'Any β HPV' category included men seropositive for at least one β HPV types.

Table A2.4. Association between grouped and type-specific cutaneous HPV seropositivity and separate categories of condyloma and suggestive of condyloma

	Condyloma[a]	Suggestive of Condyloma[b]
HPV type	**OR 95% CI**	**OR 95% CI**
Any-HPV		
Seronegative	1.00	1.00
Seropositive	1.47 (0.77 - 2.84)	0.63 (0.33 - 1.19)
Any β HPV		
Seronegative	1.00	1.00
Seropositive	1.71 (1.00 - 2.95)	1.09 (0.59 - 2.03)
α-HPV 27		
Seronegative	1.00	1.00
Seropositive	1.39 (0.61 - 3.15)	1.11 (0.41 – 3.00)
γ-HPV 4		
Seronegative	1.00	1.00
Seropositive	1.23 (0.70 - 2.16)	0.76 (0.38 - 1.53)
μ-HPV 1		
Seronegative	1.00	1.00
Seropositive	0.97 (0.54 - 1.75)	0.52 (0.24 - 1.11)
η-HPV 41		
Seronegative	1.00	1.00
Seropositive	0.79 (0.32 - 1.95)	0.69 (0.23 - 2.01)
β-HPV 5		
Seronegative	1.00	1.00
Seropositive	0.88 (0.33 - 2.35)	0.93 (0.31 - 2.76)
β-HPV 8		
Seronegative	1.00	1.00
Seropositive	0.98 (0.50 - 1.95)	1.57 (0.79 - 3.13)
β-HPV 12		
Seronegative	1.00	1.00
Seropositive	0.84 (0.24 - 2.93)	0.74 (0.17 - 3.26)
β-HPV 14		
Seronegative	1.00	1.00
Seropositive	0.66 (0.15 - 2.92)	0.00 (NE)
β-HPV 17		
Seronegative	1.00	1.00
Seropositive	1.16 (0.57 - 2.37)	1.28 (0.59 - 2.79)
β-HPV 22		
Seronegative	1.00	1.00
Seropositive	1.09 (0.36 - 3.28)	1.88 (0.67 - 5.24)
β-HPV 23		
Seronegative	1.00	1.00
Seropositive	1.12 (0.47 - 2.64)	1.8 (0.78 - 4.15)
β-HPV 24		
Seronegative	1.00	1.00
Seropositive	1.00 (0.28 - 3.53)	0.88 (0.20 - 3.92)
β-HPV 38		
Seronegative	1.00	1.00
Seropositive	0.85 (0.36 - 1.97)	1.16 (0.49 - 2.75)
β-HPV 47		
Seronegative	1.00	1.00
Seropositive	1.13 (0.54 - 2.38)	0.4 (0.12 - 1.34)
Seropositivity to 1, 2, ≥3 HPV		
Seronegative	1.00	1.00
Seropositive to 1 HPV	1.40 (0.68 - 2.91)	0.40 (0.17 - 0.92)
Seropositive to 2 HPV	1.87 (0.82 - 4.26)	0.77 (0.32 - 1.89)
Seropositive to ≥ 3 HPV	1.29 0.55 - 2.99)	0.93 (0.42 - 2.05)

a. Condyloma: A lesion with koilocytes, papillomatosis, hypergranulosis, parakeratosis and dilated blood vessels. There were 62 cases of condyloma and 352 controls. Odds ratios (OR) are unadjusted.
b. Suggestive of condyloma: A lesion without koilocytes but with one or two of the other features associated with a condyloma. These lesions were most likely early condyloma that did not show complete histological features of a fully developed condyloma. There were 47 cases of suggestive of condyloma and 352 controls. Odds ratios (OR) are unadjusted.

Table A2.5. Association between cutaneous HPV seropositivity and condyloma stratified by HPV 6 or 11 tissue DNA positivity and seropositivity to HPV 6/11.

HPV type	Condyloma Tissue DNA Positive for HPV 6/11 OR (95%CI)[a]	Condyloma Seropositivity to HPV 6/11 OR (95%CI)[b]
Any-HPV		
Seronegative	1.00	1.00
Seropositive	0.69 (0.28 - 1.70)	1.34 (0.45 - 4.05)
Any-β		
Seronegative	1.00	1.00
Seropositive	0.70 (0.32 - 1.54)	2.14 (0.81 - 5.68)
α-HPV 27		
Seronegative	1.00	1.00
Seropositive	1.23 (0.35 - 4.31)	1.30 (0.32 - 5.21)
γ-HPV 4		
Seronegative	1.00	1.00
Seropositive	0.67 (0.29 - 1.55)	0.64 (0.21 - 1.91)
μ-HPV1		
Seronegative	1.00	1.00
Seropositive	0.95 (0.39 - 2.33)	0.88 (0.29 - 2.68)
ν-HPV 41		
Seronegative	1.00	1.00
Seropositive	0.78 (0.21 - 2.97)	0.44 (0.05 - 3.67)
β-HPV 5		
Seronegative	1.00	1.00
Seropositive	1.08 (0.25 - 4.57)	2.28 (0.52 - 9.97)
β-HPV 8		
Seronegative	1.00	1.00
Seropositive	0.75 (0.30 - 1.88)	1.06 (0.35 - 3.26)
β-HPV 12		
Seronegative	1.00	1.00
Seropositive	0.12 (0.01 - 1.13)	1.05 (0.11 - 9.91)
β-HPV 14		
Seronegative	1.00	1.00
Seronegative	0.53 (0.03 - 8.69)	0.0 (NE)
β-HPV 17		
Seronegative	1.00	1.00
Seropositive	0.99 (0.36 - 2.74)	1.52 (0.48 - 4.79)
β-HPV 22		
Seronegative	1.00	1.00
Seropositive	0.64 (0.16 - 2.56)	2.28 (0.52 - 9.97)
β-HPV 23		
Seronegative	1.00	1.00
Seropositive	0.77 (0.25 - 2.37)	1.65 (0.47 - 5.80)
β-HPV 24		
Seronegative	1.00	1.00
Seropositive	0.12 (0.01 - 1.13)	2.98 (0.47 - 19.1)
β-HPV 38		
Seronegative	1.00	1.00
Seropositive	0.17 (0.05 - 0.58)	1.84 (0.51 - 6.55)
β-HPV 47		
Seronegative	1.00	1.00
Seropositive	1.23 (0.35 - 4.31)	1.63 (0.36 - 15.70)
Seropositivity to 1, 2, ≥3 types		
Seronegative	1.00	1.00
Seropositive to 1 types	1.46 (0.47 - 4.58)	1.46 (0.41 - 5.20)
Seropositive to 2 types	0.41 (0.13 - 1.28)	0.82 (0.17 - 3.86)
Seropositive to ≥ 3 types	0.48 (0.16 - 1.48)	1.73 (0.46 - 6.56)

a. Condyloma were stratified by tissue D NA positivity to HPV 6/11. Condyloma negative for DNA of 6/11 were the reference group. Out of 109 condyloma, 71 (65.14%) were positive for DNA of HPV 6/11. Odds ratios (OR) are unadjusted.

b. Condyloma were stratified by serostatus to HPV 6/11. Seronegative for HPV 6/11 were the reference group. Out of 109 men with condyloma 21 (19.27%) were seropositive for HPV 6/11. Odds ratios (OR) are unadjusted.

Table A2.6. Association between grouped and type-specific cutaneous HPV seropositivity and external genital lesions and controls examining interaction with seropositivity to 9-valent HPV vaccine types

	EGLs[a] Other than Condyloma, Suggestive of Condyloma and PeIN			Condyloma And Suggestive of Condyloma[b]			All EGL Cases[c]		
Variable	**Beta**	**Chi-square**	**P-value[f]**	**Beta**	**Chi-square**	**P-value[f]**	**Beta**	**Chi-square**	**P-value[f]**
Any-HPV[d]	-0.849	4.998	0.025	-0.014	0.003	0.960	-0.286	1.422	0.233
9vHPV[e]	-0.301	0.248	0.619	0.029	0.004	0.950	-0.143	0.128	0.721
Any-HPV*9vHPV	0.785	1.161	0.281	-0.010	0.000	0.986	0.276	0.346	0.556
Any-β HPV	-0.275	0.439	0.508	0.452	2.863	0.091	0.250	1.138	0.286
9vHPV	-0.085	0.034	0.854	0.140	0.176	0.675	0.021	0.005	0.943
Any-β HPV *9vHPV	0.584	0.734	0.392	-0.334	0.483	0.487	-0.033	0.006	0.938
α-HPV 27	0.857	2.473	0.116	0.403	0.816	0.366	0.503	1.724	0.189
9vHPV	0.214	0.354	0.552	0.058	0.051	0.822	0.078	0.122	0.727
α-HPV 27 *9vHPV	-0.607	0.472	0.492	-0.410	0.331	0.565	-0.445	0.537	0.464
γ-HPV 4	-0.779	2.360	0.124	0.010	0.001	0.973	-0.110	0.185	0.667
9vHPV	-0.133	0.105	0.746	0.021	0.005	0.942	-0.019	0.006	0.940
γ-HPV 4 *9vHPV	1.094	2.170	0.141	-0.002	0.000	0.996	0.187	0.184	0.668
μ-HPV1	-1.859	6.252	0.120	-0.180	0.373	0.541	-0.461	2.946	0.086
9vHPV	-0.084	0.049	0.825	0.113	0.163	0.687	0.041	0.029	0.864
μ-HPV 1*9vHPV	1.721	3.343	0.067	-0.308	0.323	0.570	0.066	0.020	0.888
η-HPV 41	-0.651	0.739	0.390	-0.083	0.038	0.845	-0.170	0.205	0.650
9vHPV	0.116	0.112	0.738	0.093	0.137	0.711	0.082	0.142	0.706
η-HPV 41*9vHPV	0.577	0.271	0.602	-0.786	0.777	0.378	-0.400	0.323	0.570
β-HPV 5	-0.949	0.826	0.363	-0.405	0.511	0.475	-0.396	0.673	0.412
9vHPV	0.113	0.107	0.744	-0.037	0.021	0.885	-0.004	0.000	0.985
β-HPV 5*9vHPV	0.960	0.527	0.468	0.639	0.634	0.426	0.537	0.593	0.441
β-HPV 8	-0.129	0.063	0.801	0.235	0.515	0.473	0.193	0.448	0.503
9vHPV	-0.045	0.013	0.910	0.032	0.014	0.906	0.004	0.000	0.986
β-HPV 8*9vHPV	0.730	0.914	0.339	-0.105	0.035	0.851	0.071	0.022	0.881
β-HPV 12	-12.207	0.001	0.971	-0.721	0.873	0.350	-0.711	1.195	0.274
9vHPV	-0.110	0.095	0.758	-0.033	0.017	0.896	-0.069	0.101	0.750
β-HPV 12*9vHPV	13.748	0.002	0.967	1.057	1.003	0.317	1.535	3.173	0.075
β-HPV 14	-0.037	0.001	0.973	-13.464	0.001	0.980	-0.619	0.601	0.438
9vHPV	0.117	0.116	0.733	0.015	0.004	0.950	0.031	0.022	0.883
β-HPV 14*9vHPV	0.458	0.113	0.737	13.090	0.001	0.980	0.544	0.287	0.592
β-HPV 17	-0.610	0.648	0.421	0.520	2.013	0.156	0.296	0.764	0.382
9vHPV	0.134	0.133	0.715	0.165	0.378	0.539	0.113	0.235	0.628
β-HPV 17*9vHPV	0.523	0.286	0.593	-0.821	1.848	0.174	-0.437	0.699	0.403
β-HPV 22	-0.368	0.120	0.729	0.609	1.344	0.246	0.491	1.052	0.305
9vHPV	0.115	0.112	0.738	0.063	0.063	0.802	0.063	0.084	0.771
β-HPV 22*9vHPV	0.673	0.251	0.617	-0.660	0.579	0.447	-0.437	0.340	0.560
β-HPV 23	-0.764	0.531	0.466	0.783	3.367	0.067	0.443	1.170	0.279
9vHPV	0.131	0.136	0.712	0.142	0.302	0.582	0.112	0.254	0.614
β-HPV 23*9vHPV	0.745	0.353	0.552	-1.059	2.337	0.126	-0.654	1.132	0.287
β-HPV 24	-12.186	0.001	0.977	0.205	0.088	0.766	0.092	0.022	0.883
9vHPV	0.154	0.212	0.645	0.056	0.051	0.821	0.071	0.112	0.738
β-HPV 24*9vHPV	11.845	0.001	0.978	-0.579	0.296	0.586	-0.476	0.262	0.608

(Continued on next page)

Table A2.6. (Continued)

	EGLs[a] Other than Condyloma, Suggestive of Condyloma and PeIN			Condyloma and Suggestive of Condyloma[b]			All EGL Cases[c]		
Variable	**Beta**	**Chi-square**	**P-value[f]**	**Beta**	**Chi-square**	**P-value[f]**	**Beta**	**Chi-square**	**P-value[f]**
β-HPV 38	0.497	0.879	0.349	0.176	0.182	0.669	0.228	0.412	0.521
9vHPV	0.241	0.445	0.505	0.099	0.147	0.701	0.114	0.260	0.610
β-HPV 38*9vHPV	-0.577	0.444	0.505	-0.513	0.564	0.452	-0.500	0.747	0.388
β-HPV 47	-0.729	0.931	0.335	-0.478	1.044	0.307	-0.468	1.381	0.240
9vHPV	0.187	0.288	0.591	-0.041	0.025	0.875	0.018	0.007	0.935
β-HPV 47*9vHPV	0.170	0.024	0.877	0.552	0.663	0.415	0.338	0.323	0.570
Seroactivity to 1 HPV	-0.688	2.531	0.112	-0.262	0.606	0.436	-0.466	2.708	0.100
Seroactivity to 2 HPV	-1.412	3.314	0.069	0.304	0.633	0.426	-0.103	0.090	0.764
Seroactivity to ≥ 3 HPV	-0.843	2.037	0.154	0.115	0.091	0.763	-0.126	0.146	0.702
9vHPV	-0.301	0.248	0.619	0.029	0.004	0.951	-0.143	0.128	0.721
Seroactivity to 1 HPV*9vHPV	-0.283	0.079	0.778	0.208	0.105	0.745	0.268	0.231	0.631
Seroactivity to 2 HPV *9vHPV	1.399	1.532	0.216	-0.317	0.189	0.663	0.089	0.020	0.888
Seroactivity to ≥3 HPV *9vHPV	1.290	2.034	0.154	-0.111	0.027	0.870	0.301	0.273	0.601

a. This category is also referred to as 'other EGL cases' included different other diagnoses i.e. molluscum contagiosum, intradermal nevus, fibroepithelial polyp (skin tag), chronic balanitis, genital melanotic macule, psoriasiform dermatitis, lichenoid tissue reaction, and acute mucositis.

b. Condyloma: A lesion with koilocytes, papillomatosis, hypergranulosis, parakeratosis and dilated blood vessels. Suggestive of condyloma: A lesion without koilocytes but with one or two of the other features associated with a condyloma. These lesions were most likely early condyloma that did not show complete histological features of a fully developed condyloma.

c. This category included all pathological diagnoses of EGLs.

d. Any-HPV variable included seropositivity to at lease of the 14 cutaneous HPV, β types: 5, 8, 12, 14, 17, 22, 23, 24, 38, and 47, α type 27, γ type 4, μ type 1, and ν type 41 assessed in this study.

e. 9vHPV variable included seropositivity to at least of the 9-valent HPV (9vHPV) vaccine types: 6, 11, 16, 18, 31, 33, 45, 52, and 58.

f. Logistic regression was used to obtain Wald Chi-square and p-values. Reference categories included seronegative for each HPV types and control group. P-values for interaction terms are shaded and significant p-values are highlighted in bold.

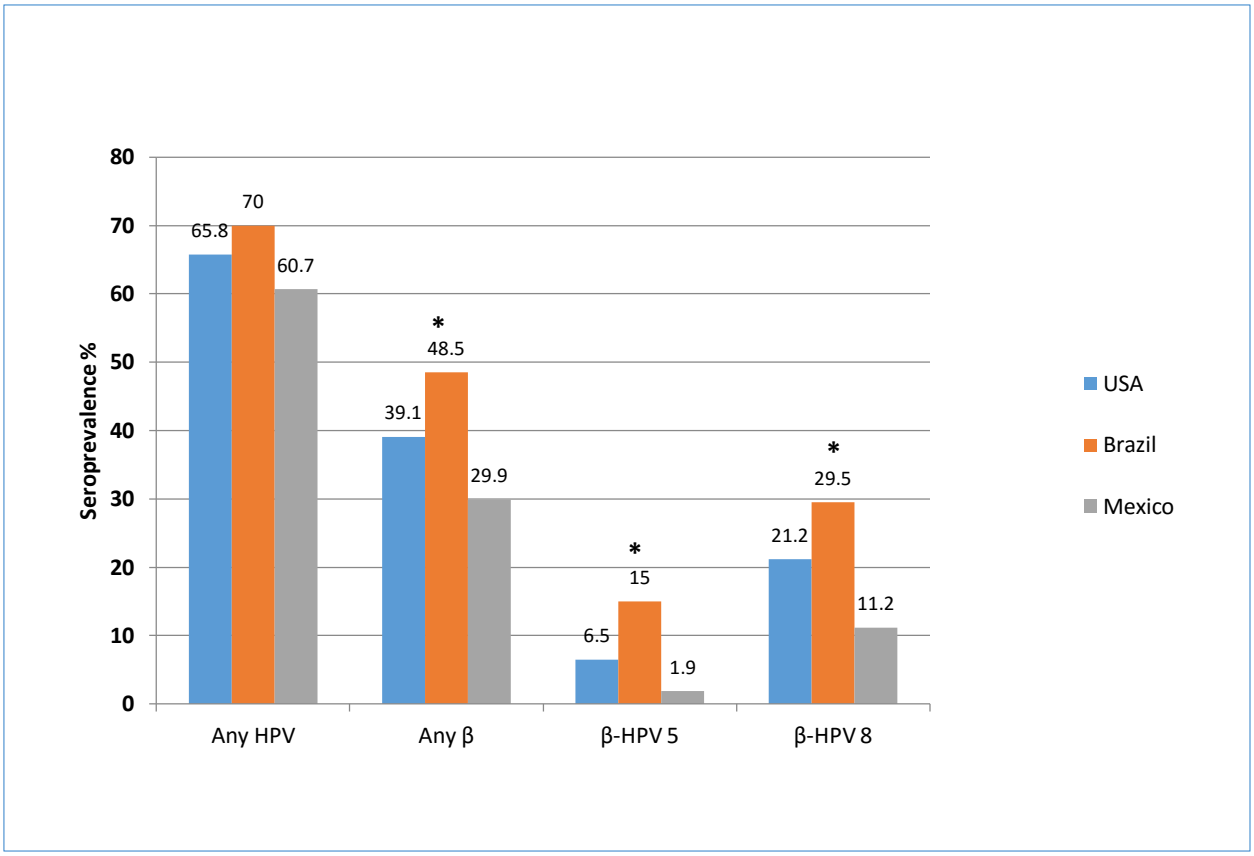

Figure A2.1. Seroprevalence of grouped and type-specific cutaneous HPV at baseline of men residing in the U.S. Brazil and Mexico

Any-HPV variable was created if a persons tested positive for ≥1 of the 14 cutaneous HPV types included in this study, he was considered seropositive for any-HPV. Any-β variable was created if a person tested positive for ≥ 1 of the 10 beta HPV types included in this study, he was considered seropositive for any-β HPV. P-value is from Chi-square test. Except for 'any-HPV' category, seroprevalence significantly differed ($p<0.05$) by country in all other categories. * Denotes significant p-value.

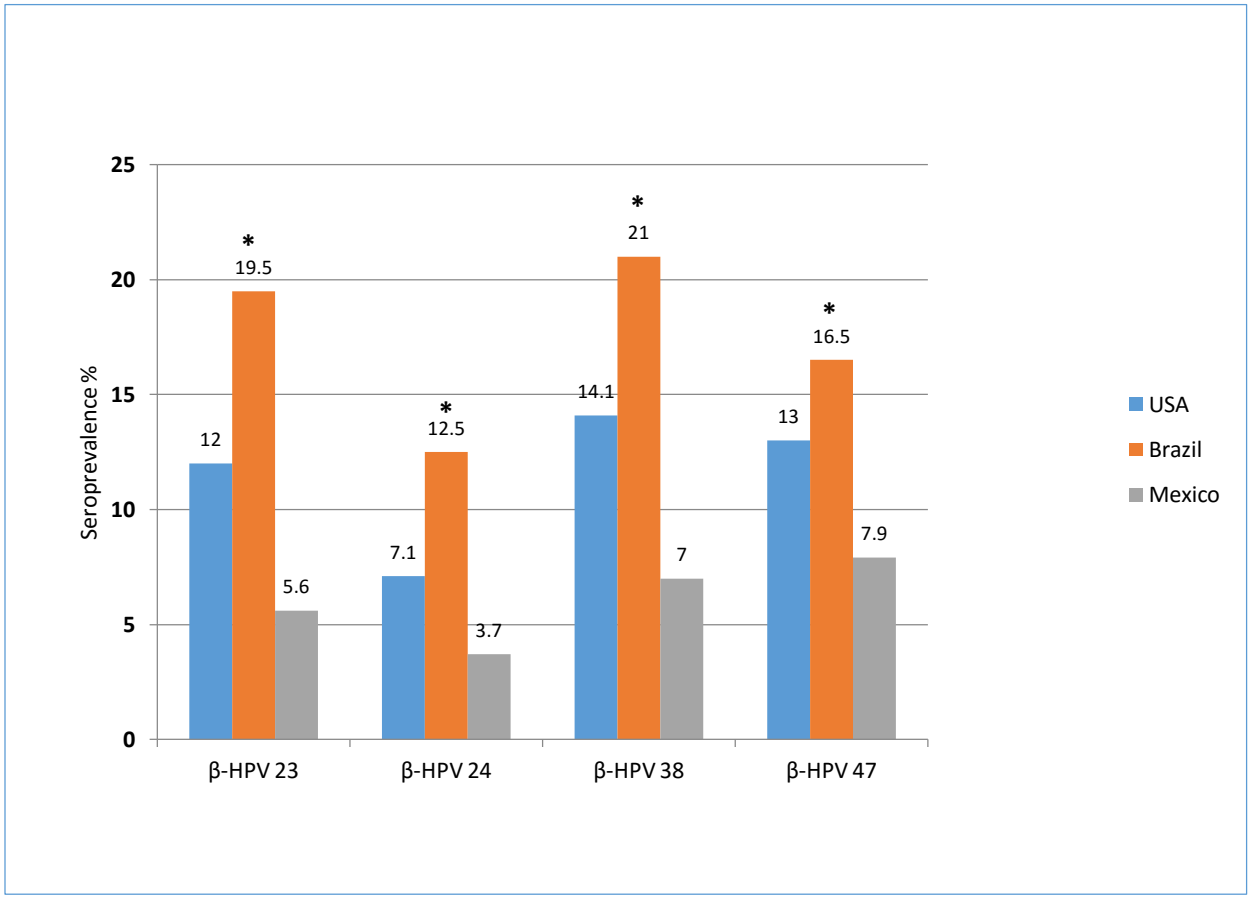

Figure A2.2. Seroprevalence of type-specific cutaneous HPV at baseline of men residing in the U.S. Brazil and Mexico

P-values is from Chi-square test. Seroprevalence for all individual HPV types significantly differed ($p<0.05$) by country. * Denotes significant p-value.

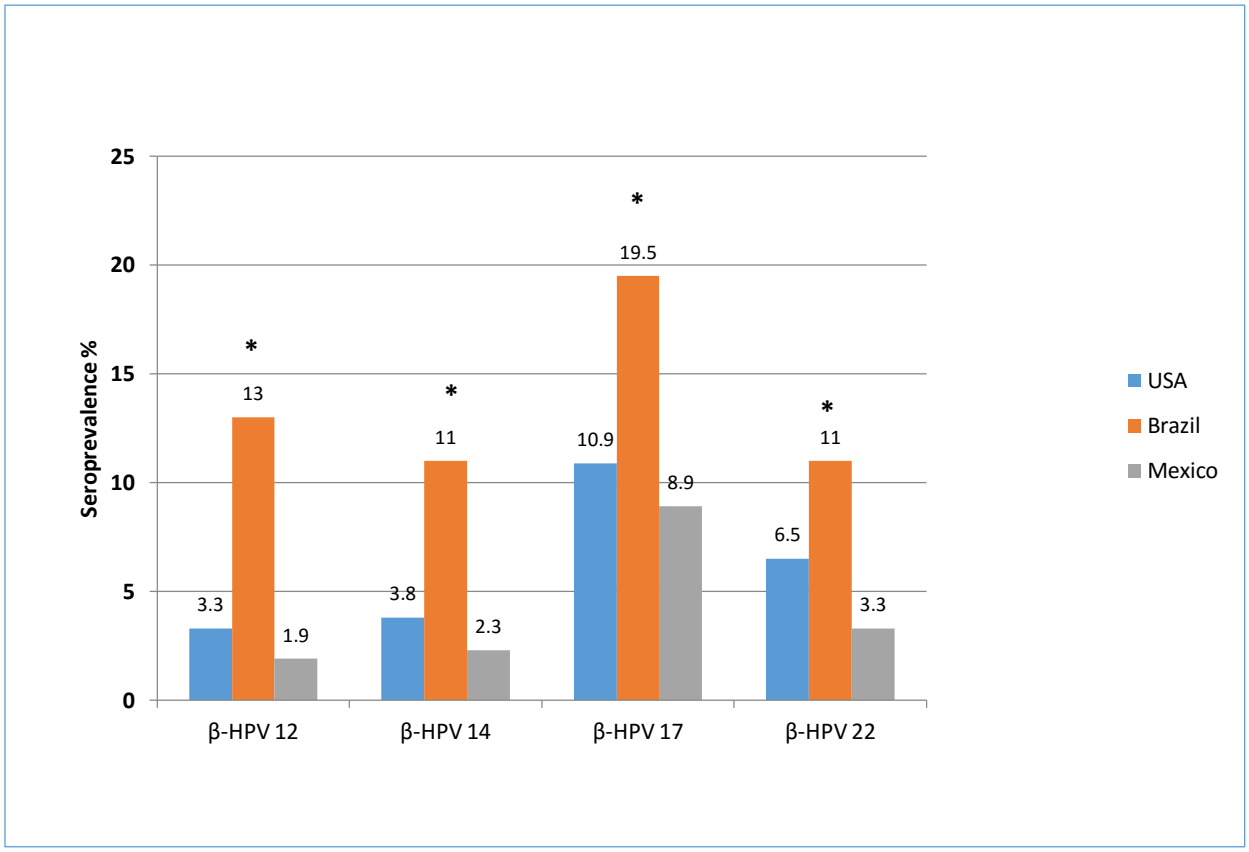

Figure A2.3. Seroprevalence of type-specific cutaneous HPV at baseline of men residing in the U.S. Brazil and Mexico

P-values is from Chi-square test. Seroprevalence for all individual HPV types significantly differed ($p<0.05$) by country. * Denotes significant p-value.

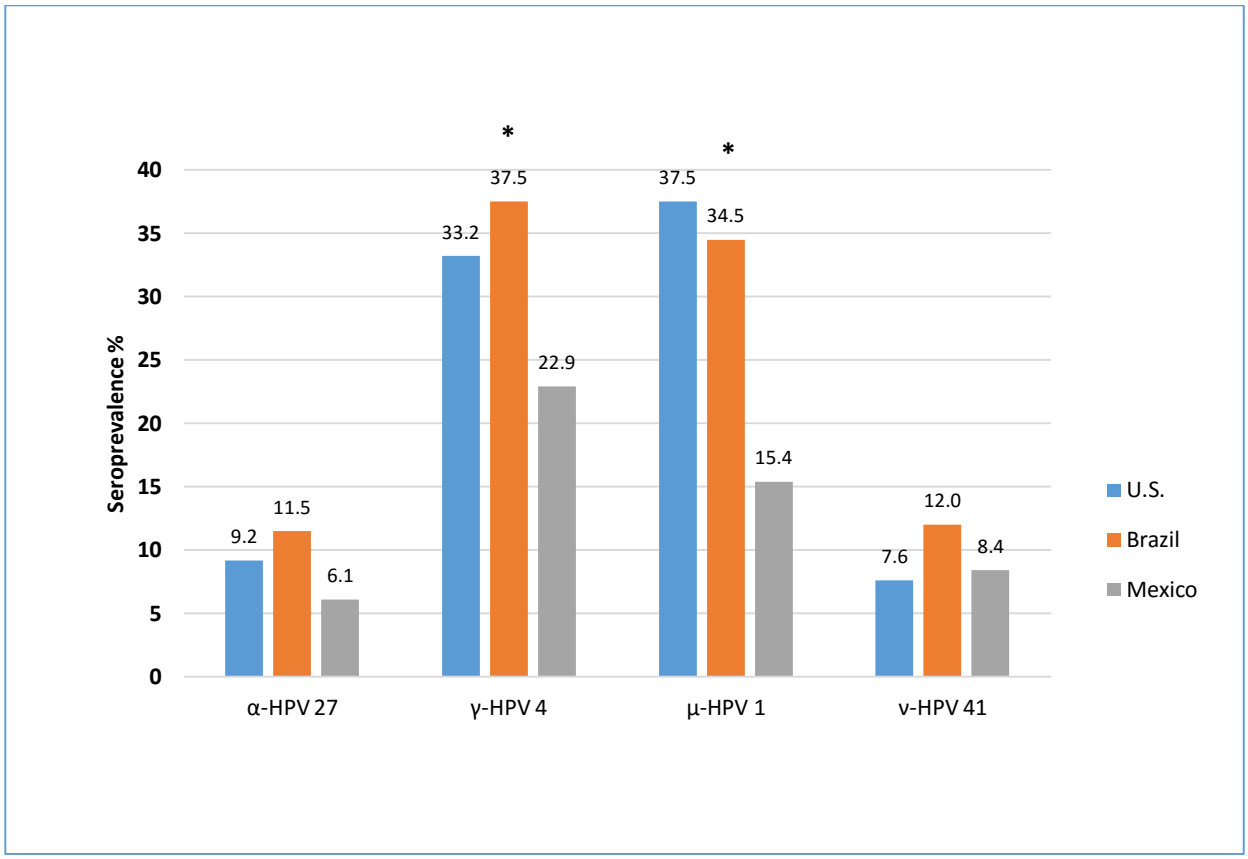

Figure A2.4. Seroprevalence of type-specific cutaneous HPV at baseline of men residing in the U.S. Brazil and Mexico

P-values is from Chi-square test. Seroprevalence for γ-HPV 4 and μ-HPV 1 significantly differed ($p<0.05$) by country.
* Denotes significant p-value.

ABOUT THE AUTHOR

Shams Rahman was born in Afghanistan in 1980, and raised in the Afghan refugee camps in Pakistan. He received his MD degree from Kabul Medical University in 2004, and his MBA degree in Health Management from Preston University Pakistan in 2006. Shams has worked in senior managerial positions for the United Nations, Johns Hopkins University, and other international non-for-profit organizations, primarily in the primary healthcare research and community development projects in Afghanistan. He specializes in monitoring, evaluation and impact assessments. He has also worked as an independent consultant for numerous organizations such as Department for International Development (DFID) UK, Dutch Ministry of Foreign Affairs, Japanese International Cooperation Agency (JICA), Future Generations U.S., American Friends Service Committee/Quakers U.S., and the Dutch Development Organization (Cordaid) and the Swedish Committee for Afghanistan.

In 2010, Shams received the prestigious Fulbright Scholarship to complete his advanced studies in public health in the U.S. He received his Dual MPH degree in Epidemiology and Global Health from the University of South Florida in 2013, and subsequently completed his PhD in Public Health in 2016. During his academic career, Shams has been the recipient of numerous honors and awards including two times first position in the Fulbright Scholars National Competition, USF SHARP Award, and Research Training/Education Core (RTEC) Award. He has authored and co-authored several peer reviewed articles in the scientific literature, and has presented his work at many scientific meetings, including the International HPV conference and the American Public Health Association (APHA) annual meetings. Shams serves as the editor-in-chief of the Afghanistan Journal of Public Health (AFJPH) and remains an active member of several other public health organizations.