Human Papillomavirus and Genital Disease in Men: What We Have Learned from the *HIM Study*

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Keywords

Cohort study · HPV · Men · PCR

Abstract

It is currently recognized that in addition to the major impact of human papillomavirus (HPV) infection in females, HPV causes considerable disease in men at the genitals, anal canal, and oropharynx. Specifically, genital HPV infections may progress to genital warts and penile carcinoma. Although studies concerning the natural history of HPV infections and associated neoplasias have mainly focused on women, during the last 2 decades considerable attention has been given in further understanding these infections in men. The HIM (HPV infection in men) Study, the only prospective multicenter study of male HPV natural history, consisted of a large prospective international cohort study in which men from Brazil, the United States, and Mexico were enrolled. The design and protocols of this study allowed unraveling crucial information regarding the relationship between HPV infection and clinical consequences in men, and associated risk factors at each of the anatomic sites where HPV is known to cause cancer in men.

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Introduction

Human papillomaviruses (HPVs) are non-enveloped, 50 nm viruses that enclose a circular double stranded DNA of about 8,000 base-pair, physically divided into 3 regions: the early (E) and late (L) regions and the long control region. While E1 and E2 proteins play crucial roles in viral DNA transcription and replication regulation, E4, E5, E6, and E7 proteins are involved in cell cycle deregulation, immune evasion, and recruitment of replication host factors [1]. L1 and L2 late proteins constitute the major and secondary capsid proteins respectively. Finally, the long control region is a noncoding region that encloses a plethora of cis-elements crucial for viral early transcription and replication regulation [2].

HPVs infect the stratified squamous epithelia, both mucosal and cutaneous, and preferentially target mitotically active cells of the basal layer. The E6 and E7 proteins of oncogenic types of HPV stimulate cell proliferation by interacting directly, in addition to several other cellular proteins, with p53 and pRB proteins, respectively, both master regulators of the cell cycle [1]. The consequence is

expansion of the life span of infected cells favoring the accumulation of mitotic defects, genomic instability and finally neoplasia development.

HPVs are a heterogeneous group of epitheliotropic viruses that belongs to the *Papillomaviridae* family [3]. HPVs are classified taxonomically based on nucleotide sequence identity, and by now over 200 types have been fully characterized, most of which cluster phylogenetically within the Alpha (α)-, Beta (β)-, or Gamma (γ)-HPV genus [4]. While the majority of α -HPVs are mucosal types isolated from the anogenital epithelia, some types of this genus, in addition to all β - and γ -HPVs, are originally designated “cutaneous types.” The International Agency for Research on Cancer has classified 13 α -HPVs as group 1 carcinogens (-16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59 and -66), commonly referred to as “high-risk HPVs” (HR-HPVs), among which HPV-16 is undoubtedly the most carcinogenic based in the burden of cancers and cancer precursors lesions associated to this infection [3]. On the other hand, HPVs-6, -11, -40, -42, -43, -44, and -54 are grouped as “low-risk (LR)-HPVs” due to epidemiological association with benign proliferations such as condylomas acuminata. Nevertheless, while classified as LR, HPV-6 has been identified in several malignancies, including carcinomas of the vagina [5], vulva [6, 7], penis [8], tongue [9], cervix [10, 11], and tonsils [12].

Although studies concerning the natural history of HPV infections and associated neoplasias have mainly focused women, an increasing interest in further understanding the relationship between HPV and disease in men has emerged during the last 2 decades. In fact, it is recognized that HPV is mostly transmitted sexually, and infection by these viruses is strongly linked to the development of tumors in the cervix, vulva, and vagina in women, cancer of the penis in men, as well as tumors in the anal canal and head and neck (predominantly the oropharynx) in both genders.

HPV-Associated Genital Disease in Men

HPV infection in the penis is detected in 16–69% of healthy men depending on the population studied, the anatomical site of sampling, and the laboratory method used for HPV detection and typing [13]. These infections may progress to external genital lesions (EGLs), mostly condyloma acuminata (condyloma or genital warts [GW]) and penile intraepithelial neoplasia (PeIN) that are thought to precede penile cancer [14].

GW is a common clinical outcome of HPV infection in males and affects mainly individuals aged 25–29 years, decreasing with age; however, the incidence of condyloma remains relatively high throughout a man’s life span [15–20]. LR-HPVs 6 and 11 are the etiologic agents of over 90% of GWs [3]; however, in approximately one-third of the cases multiple viral types, including coinfection with HR-HPV, are detected [21]. GWs are highly infectious and about 65% of the individuals who have sex with an infected partner will also develop GWs [22]. Most lesions develop within 2–3 months after an HPV infection [20, 23], and although about 30% of these warts spontaneously regress, recurrence is common, and therefore, treatment impacts in high medical costs [24, 25]. The *HIM Study* is the only study to document the high rate of GW recurrence under conditions of careful repeated clinical examinations of men for up to 7 years of follow-up [26]. Additionally, even if GW is a benign condition, not associated with mortality, it is an important cause of psychosocial distress and physical discomfort [27].

Penile cancer is a rare devastating disease that accounts for less than 0.5% of all cancers in men globally [28], and affects mostly individuals aged 50–70 years [29]. Worldwide, areas with high incidence of cervical cancer also tend to have a high incidence of penile cancer [30]: incidence rates are higher in less developed countries representing up to 10% of male tumors in some parts of Africa, Asia, and South America, in contrast to the United States and Western Europe in which it represents around 0.4% of all malignancies [31]. The etiology of penile cancer is multifactorial and several risk factors have been identified, including phimosis, poor hygiene, smoking, and chronic inflammatory states, in addition to early age at first sexual intercourse, high number of sexual partners, lack of condom use and neonatal circumcision, and history of GWs [32, 33]. Furthermore, ~50% of all penile cancers appear to be attributable to HPV infection with HR-HPV-16 being the most commonly detected type [34, 35]. Whether HPV-related penile cancers have an improved survival profile compared with cancers unrelated to HPV infection is still a matter of debate [36].

The HIM (HPV in Men) Study

With the aim of increasing the knowledge concerning HPV infection in men (HIM), a large prospective international cohort study, the HIM Study, was conducted. This study was unique in comparing the natural history of HPV

in men from the United States, Mexico, and Brazil representing low- and high-risk countries for penile cancer. This study was funded by the National Institute of Health (NIH-USA) to Anna R Giuliano (H. Lee Moffitt Cancer Center and Research Institute, USA) as the Principal Investigator, with Luisa Lina Villa (Instituto do Cancer do Estado de Sao Paulo, Brazil), and Eduardo Lazcano-Ponce (National Institute of Public Health in Cuernavaca, Mexico) as co-Principal Investigators. Eligibility criteria for participation in the study included (a) ages between 18 and 44 years and another group between 45 and 70; (b) living in South Florida, the United States, Sao Paulo, Brazil, or the State of Morelos, Mexico; (c) no previous diagnosis of penile or anal cancer; (d) no diagnosis of genital or anal warts at admission; (f) not HIV infected or have AIDS at diagnosis; and (e) agreement with the scheduled visits every 6 months planned initially for 4 years.

In Mexico, men were recruited at the Instituto de Medicina y Seguridad Social of the State of Morelos. In Brazil, men were recruited at the Reference Center for Treatment of Sexually Transmitted Diseases and AIDS (CRT-AIDS) in São Paulo and from the general population. In the United States, participants came from the University of South Florida and Tampa metropolitan area.

Scheduled visits consisted of filling out a questionnaire, undergoing a clinical examination that included visual inspection of the skin and external genitalia, and the collection of blood sample, mouth wash, and genital (coronal sulcus, penis glands and shaft, and scrotum) and anal canal swabs. In the cases of men presenting EGLs, tissue samples were further collected for diagnosis and possible treatment referral. In genital, anal, and oral samples, HPV detection testing was performed by PCR employing the PGMY09/11 consensus primers [37], followed by typing using the Linear Array HPV genotyping test (Roche Molecular Diagnostics, CA, USA) capable of discriminating 37 α -HPV types commonly detected at the cervix [38]. It is important to highlight, that although this review focuses data regarding the male genitals, the HIM Study reported HPV infection and natural history information also for the anal canal and oral anatomical sites.

HPV Genital Infection and Associated Diseases: The HIM Study

HIM participants were recruited from September 2005 to September 2009 with a total of 4,292 men: 1,443 from Brazil, 1,426 from the United States, and 1,423 from Mexico. Furthermore, a group aged above 45 years was com-

posed of about 180 men from each center [39]. Baseline analysis of this cohort revealed that genital HPV infection prevalence is higher among asymptomatic men than previously described for women [40]; HPV infection was detected in 65.2% men including 12% by HR-HPVs only, 20.7% by LR-HPVs only, 17.8% of multiple infections by HR- and LR-HPVs, and 14.7% of infections were categorized as “unclassified infection” [39] (Table 1). Particularly among virgin men, the prevalence of any and HR-HPVs was 25 and 18.2% respectively [59], which points toward the transmission of infection outside of penetrative sexual intercourse and vertical transmission.

When data were analyzed separately by geographic region, HPV infection prevalence was higher among men recruited in Brazil (72.3%) compared to that in the United States (61.3%) and Mexico (61.9%) [39]. HPVs 16, 51, and 59 were the most commonly detected HR-HPV types, and HPV 84, 62 and 6 were the most prevalent LR-HPVs, although some regional differences were observed regarding the distribution of viral types. Furthermore, the prevalence and incidence of the predominant disease-causing genital HPVs infections in males (HPVs 16 and 6) were also significantly higher among men from Brazil than men from Mexico [63]. It is important to mention that solely discrete differences in the burden of genital HPV infection among MSM (men who have sex with men) and MSW (men who have sex with women) in all 3 countries analyzed were observed [57].

“Unclassified HPV” infections were more commonly detected among men recruited in the USA (20%), as compared to Mexico (13.5%) and Brazil (10%), and significantly more prevalent among younger men [39]. These infections include HPV PCR-positive specimens for which hybridization to any type-specific probe was not observed. However, using a very sensible bead-based multiplex HPV genotyping methodology, a high prevalence of cutaneous β -HPVs was observed in most of these specimens [50, 54, 58, 62]. The lack of association between β -HPVs DNA detection and sexual behavior variables suggested other routes of transmission of these infections such as autoinoculation [54, 58]. Nevertheless, concordance of β -HPVs across different anatomic sites assessed was low [62], although higher across keratinized tissue sites (31.0%; genital skin, eyebrow hairs, forearm skin) compared to mucosal sites (6.9%; anal and oral mucosa) [60]. The clinical consequences of the presence of β -HPVs at the male genitals require further research. Of note, among HIM participants, age and blistering sunburn were significant risk factors for β -HPVs detection in normal skin swabs and eyebrow hairs [65].

Table 1. Main findings regarding the prevalence, incidence, and persistence of HPV infections at the male genitals in the *HIM Study*

Year	Author(s) (ref)	Main findings
2008	Giuliano et al. [39]	Genital HPV prevalence was 65.2% (12% HR-HPVs only, 20.7% LR-HPVs only, 17.8% both LR- and HR-HPVs, 14.7% unclassified HPV infections) Multiple HPVs were detected in 25.7% of men Genital HPV was more prevalent in Brazil than in the United States and Mexico HPV prevalence was not associated to age
2009	Giuliano et al. [41]	Any-HPV infection was associated with race, lifetime, and recent number of sexual partners, having sex in the past 3 months HR-HPV was associated with lifetime and recent number of sexual partners, having sex in the past 3 months LR-HPV infection was associated with a lifetime number of sexual partners
2011	Giuliano et al. [42]	Incidence of a new genital HPV infection was 38.4 per 1,000 person month Median duration of HPV infection was 7.5 months for any-HPV and 12.2 months for HPV-16 Clearance of HR-HPV infection decreased in men with a high number of lifetime female partners Clearance of HR-HPV was associated to age
2011	Nyitray et al. [43]	Prevalence of genital HPV may be higher among MSWM than among MSW or MSM
2012	Akogbe et al. [44]	Asian/Pacific Islanders had the lowest HPV prevalence compared to Blacks and Whites
2012	Repp et al. [45]	HPV prevalence was high even among those who reported always using condoms
2012	Schabath et al. [46]	Any- and HR-HPV was associated to smoking, although only evident among men reporting fewer lifetime sexual partners
2013	Alberts et al. [47]	HPV detection was associated to <i>Chlamydia trachomatis</i> infection among men reporting ≥ 2 recent sexual partners HPV detection was associated to HSV-2 serostatus among men reporting 0–5 lifetime sexual partners
2013	Schabath et al. [48]	HPV incidence and acquisition were lower among men of Asian/Pacific Islanders race
2013	Pierce-Campbell et al. [49]	No protective effects of condom use were observed among monogamous men The risk of HPV acquisition was twofold lower among men with no steady sex partner who always used condoms, compared with those who never used condoms The probability of clearing an oncogenic HPV infection was 30% higher among nonmonogamous men who always used condoms
2013	Sichero et al. [50]	Cutaneous β - and γ -HPV were very prevalent at the male genitals
2013	Albero et al. [51]	HPV infection was not associated to men circumcision
2014	Schabath et al. [52]	HPV incidence and acquisition were higher among current smokers
2014	Albero et al. [53]	Except for specific viral types, HPV incidence and clearance was not associated to men circumcision
2014	Sichero et al. [54]	β -HPV infections were not associated to any of the risk factors accessed
2015	Schabath et al. [55]	Prevalent HPV infections were associated with high intake of alcohol
2015	Ingles et al. [56]	Men remained susceptible to HPV infections throughout their lifespan
2015	Nyitray et al. [57]	Genital HPV natural history may be similar in HIV-negative MSM and MSW
2016	Nunes et al. [58]	β -HPV infection was not associated to variables regarding sexual behavior
2016	Liu et al. [59]	Any-HPV and HR-HPV prevalence was 25 and 18.2% among male virgins respectively
2017	Hampras et al. [60]	Concordance of any β -HPV infection was higher across keratinized tissue sites compared to mucosal sites
2017	Ranjeva et al. [61]	Infection with one HPV type strongly increased the risk of infection with the same type for years afterward

Table 1 (continued)

Year	Author(s) (ref)	Main findings
2017	Nunes et al. [62]	Concordance of β -HPVs across sites was low and mainly observed among paired genital/anal canal samples β -HPV type-specific concordance seem to occur more often than would be expected by chance
2017	Sudenga et al. [63]	HPVs 16 and 6 prevalence and incidence were higher among men from Brazil than men from Mexico
2018	Liu et al. [64]	Rates of any-HPV incident infection was 26.2 and 14.6 per 1,000 person-months among virgins initiating sex (VIS) and virgins not initiating sex (VNIS) during follow-up, respectively After penetrative sex initiation, 45.5% of the men acquired HPV within 24 months
2018	Pamnani et al. [26]	Recurrence of HPV infections was common and associated with high-risk sexual behavior

HR, high-risk; LR, low-risk; MSW, men who have sex with women; MSM, men who have sex with men; MSWM, men who have sex with women and men; HSV, herpes simplex virus; HIV, human immunodeficiency virus.

The incidence of a new HPV infection, considering a 12-month period along the *HIM Study*, was 38.4 per 1,000 person-months [42]. Furthermore, the 12-month cumulative incidence was similarly high among both MSM and MSW in all 3 populations studied [57]. Interestingly, for HPV-16, an initial infection increased the 1- and 2-year probability of reinfection by 20 and 14-fold respectively, in both sexually active and celibate men [61], suggesting that reinfection may originate from autoinoculation or episodic reactivation of latent virus.

Another important point regarding the natural history of HPV infection regards persistence (defined as detection of HPV DNA of a specific type at ≥ 2 consecutive visits). Data from the *HIM Study* revealed that HPV infections are less prone to be persistent in men than in women: in men, the median time to clearance of any-HPV infection was 7.5 months, with 66 and 90% of infections clearing within 12 and 24 months respectively [42], and was similar between MSM and MSW [57]. In addition, it was observed that HPV-16 infections were more likely to have a longer duration, twice as long to clear compared to HPV-18 and other HPV types [42].

At each study visit, HIM participants answered an epidemiologic questionnaire that included several queries regarding sexual behavior and personal habits, which allowed for an evaluation of a number of factors associated with male genital HPV infection and persistence. HPV infection prevalence and incidence in men varied by race, with Asian/Pacific Islanders having the lowest rates compared to Blacks and Whites [41, 44, 48]. HR-HPV infection prevalence and incidence were also higher among current smokers [46, 52], and individuals reporting high alcohol intake [55].

Regarding age, while HPV prevalence is highest among 14–24 years old women with a decrease until middle age [66], there was no clear association between age and genital HPV prevalence in men, regardless of the country examined [39]. Furthermore, the rates of detection of incident genital HPV infections [42], and the likelihood of clearing HPV types 16 and 6 infections [56], remained fairly constant across age groups. Altogether, it appears that men remain at risk for prevalent genital HPV infection across the life span, and that HPV infection and clearance may differ by gender.

A positive association between genital HPV detection in men and measures of sexual history with both male-anal and female partners, including lifetime and recent (last 3 months) number of sexual partners and sexual frequency was observed [41, 42]. Genital HPV prevalence was typically higher among MSWM (men who have sex with women and men) than among MSM or MSW for groups of HPV genotypes including LR-HPVs (51, 36, and 42% respectively), and multiple HPV types (37, 24, and 29% respectively) [43]. In fact, lifetime number of sexual partners was the most significant risk factor for the acquisition of genital HPV infection. The incidence rate for any-HPV infection among virgins initiating sex and virgins not initiating sex during follow up were 26.2 and 14.6 per 1,000 person-months respectively [64]. After penetrative sexual intercourse initiation, 45.5% of the men acquired HPV within 24 months. Furthermore, not only infection recurrence [26] but also HPV clearance [42, 43] was associated with high-risk sexual behavior.

Male circumcision was not associated with prevalence, incidence, or clearance of genital HPV detection [51, 53]. Nevertheless, it needs to be highlighted that the use of a

Table 2. Main findings regarding HPV-associated male genital external lesions in the *HIM Study*

Year	Author(s) (ref)	Main findings
2011	Anic et al. [67]	GW incidence was higher among men aged 18–30 years HPVs 6, 11, and 16 were the most commonly detected in GWs HPVs 6 and 11 play an important role in GW development, with the highest incidence and shortest time to detection among men with incident HPVs 6 and 11 infections
2012	Anic et al. [15]	GW incidence was associated to recent sexual behavior GWs development may be protected by condom use
2013	Pierce-Campbell et al. [68]	EGLs and the normal genital skin of men harbor a large number of β -HPV types β -HPVs were unrelated to EGL development
2015	Ingles et al. [69]	The cumulative incidence of any EGL was highest among men <45 years old The cumulative incidence of GW decreased significantly over time with age
2016	Sudenga et al. [20]	Most EGLs develop following infection with HPVs 6, 11, or 16
2016	Pierce-Campbell et al. [70]	β -HPV detection on the normal genital skin was not associated with the presence or development of GWs
2017	Sudenga et al. [71]	GW incidence in Brazil and the United States decreased with age GW incidence in Mexico remained constant across the lifespan PeIN incidence did not vary by country and age
2017	da Silva et al. [72]	In Brazil, HPV-positive men who developed EGLs were younger In Brazil, 4% of HPV positive infection progressed to EGL with the same HPV detected in the swab In Brazil, 15.7% of genital HPVs 6 and 11 infections progressed to GW In Brazil, median progression time to GW was higher for HPV-6 than for HPV-11 infection In Brazil, 1% of HPV-16 infections progressed to PeIN with a median progression time of 25 months
2017	Flores-Diaz et al. [73]	HPV-6 B1 variants were more prevalent in genital swabs that preceded GW development HPV-6 B1 variants were associated to an increased risk for GW
2017	Flores-Diaz et al. [74]	No particular HPV-11 variant associated to increased risk for GW development

GW, genital warts; EGL, external genital lesion; PeIN, penile intraepithelial neoplasia.

single combined genital sample enclosing the penis and scrotum for HPV DNA detection in the *HIM Study* likely limited the ability to identify a true effect of circumcision at the glans penis, the area expected to be most likely protected by this procedure. Consistent condom use appeared to reduce HPV genital burden among high-risk men [45, 49]. Nevertheless, HPV prevalence and persistence were high even among those who reported always using condoms, which could also be linked to the fact that in the *HIM Study* the combined genital sample used includes anatomical areas not covered by a condom. Finally, *Chlamydia trachomatis* infection and HSV-2 serostatus were also associated with prevalent genital HPV infection [47].

Approximately 5% of the 1,788 men who had an HPV genital infection during follow-up developed an EGL (9 PeIN and 86 GWs) [20] (Table 2). Men who tested positive for HPV at baseline had nearly 12 times the risk of developing GWs [15], with highest incidence among men aged 18–30 years [67, 69, 72]. GW incidence in Brazil and the United States decreased with age, while the incidence remained constant across the lifespan in Mexico [71]. Among *HIM Study* participants, 16 and 22% of genital HPV 6 and 11 infections developed into HPV 6 and 11 positive GW respectively [20]. Although HPV 6 (43.8%) and 11 (10.7%) were the most common types detected on GWs, a significant prevalence of HR-HPV types, including HPV 16 (9.8%) was also observed in addition to coinfection of LR- and HR-HPV [67]. The median time to

GW detection was 17.1 months; however, it was shortest among men with HPV types 6 or 11 infections (median of 7.7 months). Interestingly, while HPV-6 nucleotide variability was associated with GW development [73], there was a lack of association between any particular HPV-11 variant and increased risk of GW [74]. Recent sexual behavior was strongly associated with GW incidence, whereas frequent condom use was protective [15]. Finally, although EGLs and the normal genital skin of men harbor a large number of β -HPV types, these appear to be unrelated to EGL development in men [68, 70].

Among HIM participants, solely 0.5 and 2.0% of men with a genital HPV-16 infection developed an HPV-16-positive PeIN within the first 12 and 24 months of follow-up respectively [20]. It was further observed that the viral type influenced the rate of progression from genital HPV infection to disease: among men who developed PeIN, in 87.5%, there was more than 1 HR-HPV, and 57.1% contained HPV-16 [69]. In 1 PeIN III lesion, only HPV-6 was detected. HPV-16 infections had a relatively slow rate of progression with 50% of PeIN taking more than 19 months to be detected [20]. There were no differences by country and age for PeIN incidence [71, 72].

Conclusions

HPV causes considerable disease in men as well as women. At the male genitals, HPV is common although only a minority of infections progress to GW or PeIN.

Both the epidemiologic and biological relevance of HPV in penile cancer points to the importance and need for strategies to prevent benign as well as cancer and precancerous lesions of the male genitals. The quadrivalent HPV vaccine, capable of preventing women from HPV types 6, 11, 16, and 18 infections, has been shown to be also highly immunogenic in men age 16–26, and efficacious in preventing persistent genital and anal HPV infections, condyloma, and anal intraepithelial lesions [75, 76]. However, scarce information is still available to allow conclusions related to PeIN and penile cancer prevention. More research is needed to demonstrate the protective effect of the HPV vaccine on the prevention of penile cancer and precursor lesions, and protection against HPV-related oropharyngeal cancer. The *HIM Study* has clearly shown that men have a high HPV infection and related disease burden and that the epidemiology of these infections in men is different than what has been reported among women at the cervix. Altogether, the *HIM Study* data point to the benefit of gender neutral vaccination to directly benefit men as well as to more rapidly achieve population level declines in HPV infection and related diseases among both men and women.

Disclosure Statement

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