Vol. **16** • No. **1** • March **2009**

Journal of ANDROLOGICAL SCIENCES

Official Journal of the Italian Society of Andrology

Editorial Office
Lucia Castelli (Editorial Assistant)
Tel. 050 3130224 • Fax 050 3130300
lcastelli@pacinieditore.it

Eleonora Lollini (Editorial Secretary)
Tel. 050 3130283 • Fax 050 3130300
elollini@pacinieditore.it

Pacini Editore S.p.A. • Via A. Gherardesca 1
56121 Ospedaletto (Pisa), Italy

Publisher
Pacini Editore S.p.A.
Via A. Gherardesca 1,
56121 Ospedaletto (Pisa), Italy
Tel. 050 313011 • Fax 050 3130300
Info@pacinieditore.it
www.pacinimedicina.it

PACINI EDITORE MEDICINA

www.andrologiaitaliana.it

SIA

INDEX

HPV infection and the risk of penile cancer

M. Pow-Sang, J. Astigueta*

Urology Department, Instituto Nacional de Enfermedades Neoplasicas, Lima, Peru;
** Urology Department, Instituto Regional de Enfermedades Neoplasicas, Trujillo, Peru*

Primary malignant penile cancer is a rare disease. Penile cancer incidence varies among different populations, and is rare in most developed nations. In the United States, age-standardized incidence rates range from 0.3 to 1.8/100,000 [1]. Higher incidence rates are seen in underdeveloped countries, such as in Uganda (2,8/100,000), and in areas of Brazil (range from 1.5-3.7/100,000 inhabitants); the lowest incidence world-wide is reported in Israeli Jews (0.1/100,000 inhabitants) [1].

Penile cancer most commonly affects men between 50 and 70 years of age [2-4]. Younger individuals are also affected; approximately 19% of patients are less than 40 years of age [3] and 7% are less than 30 years [3 5].

Human papillomavirus (HPV) infection is the necessary etiologic agent for cervical carcinogenesis, with HPV infection in men significantly contributing to infection and subsequent cervical disease in women as well as to disease in men [6-8].

Many studies suggest an association between human papillomavirus (HPV) infection and penile cancer. The mechanism by which HPV leads to malignant transformation is likely mediated through two viral genes, E6 and E7, which are actively transcribed in HPV infected cells. The most recognized target of HPV E6 protein is TP53 [9], whereas the primary target of HPV E7 protein is RB1 and the related pocket proteins, p107 and p130 [10]. The E6 and E7 proteins bind to and inactivate the host cell's tumor suppressor gene products p53 and pRb (retinoblastoma gene) both of which are known negative regulators of cellular proliferation, leading to uncontrolled growth [11]. In cervical carcinogenesis, recombination between HPV and chromosomal DNA is frequent and likely necessary for progression, and DNA hypermethylation – specifically of the L1 gene – is a biomarker for cancerous progression [12]. Recently, Kalantari et al. [13] compared penile and cervical carcinoma with HPV 16 and HPV 18. They found numerous striking similarities: high HPV 16 methylation rates in penile carcinomas resemble those reported in cervical malignant lesions. They proposed that both penile and cervical carcinomas depend on chromosomal recombination as a necessary step in the etiological process. Their data support the causality of HPV infection in the etiology of penile cancer

Corresponding author:
Mariela Pow Sang, Assistant, Department of Urology, Instituto Nacional de Enfermedades Neoplasicas, Lima, Peru – E-mail: mrpowsang@hotmail.com

and suggest similar etiological and epidemiological parameters for HPV dependent cervical and penile carcinogenesis.

Partridge et al. recruited 240 male students, 18 to 20 years of age, at the University of Washington in Seattle to participate in a longitudinal natural history study of HPV infection [14]. At 24 months, the cumulative incidence of new infection of any genital HPV type was 62.4% (95% CI = 52.6-72.2%). The most commonly detected types were HPV-84 and HPV-16. In multivariate analysis, a report of a new sex partner during the prior 0-4 (hazard ratio [HR] = 2.0; 95% CI = 1.3-3.0) and 5-8 (HR = 1.8; 95% CI = 1.2-2.7) months and a history of smoking (HR = 1.6; 95% CI = 1.1-2.4) were associated with an elevated risk of HPV acquisition.

In men, productive HPV infection can result in simple condyloma acuminata, giant condyloma, or Buschke-Lowenstein tumor, mainly caused by PHV 6 and 11 [15]. HPV-associated penis intraepithelial neoplasia are found in the great majority of cases, but they are inconspicuous lesions caused by high-risk HPV types, especially HPV 16 and 18, histologically showing low, moderate, or severe dysplasia (PIN grades 1, 2 and 3) [16]. Less frequently, high-risk HPV infection can progress to penile carcinoma, also associated with HPV 16 in 16 to 100% of the cases, and HPV 18 in 1 to 55% of the cases [17].

In a systematic review of the literature, Dunne et al. [18] found a wide range (1-73%) of genitourinary HPV prevalence among men worldwide; 15 (56%) of these studies reported a prevalence of > 20%, which is similar to the HPV prevalence found among women (27%) [19]. Weaver et al. evaluated the distribution of HPV in men. Of 1323 samples tested (from 317 men), 215 (16%) were found to be positive for HPV DNA, including 28% from the foreskin, 24% from the penile shaft, 17% from the scrotum, 16% from the glans, and 6% from urine [20]. According to Giuliano et al., in heterosexual men, HPV detection was highest at the penile shaft (49.9%), followed by the glans penis/coronal sulcus (35.8%) and scrotum (34.2%). Detection was lowest in urethra (10.1%) and semen (5.3%) samples [21]. Nielson et al. tested 463 men for HPV at the glans/corona, penile shaft, scrotum, urethra, perianal area, anal canal, and in a semen sample. HPV testing by PCR and reverse line blot genotyping for 37 types was conducted on each of the specimens from the seven sampling sites [22]. When HPV results from any sampling site were considered, 237 (51.2%) men were positive for at least one oncogenic or nononcogenic HPV type, and another 66 (14.3%) men were positive for an unclassified HPV type. The types with the highest prevalence were HPV-16 (11.4%) and 84 (10.6%). External genital samples (glans/corona, shaft, and scrotum) were more likely than anal samples to contain oncogenic HPV (25.1% *vs*. 5.0%). HPV-positive penile shaft and glans/corona samples were also more likely to be infected with multiple HPV types than other sites.

A recent study reported the prevalence of HPV DNA in samples collected from exfoliated cells in men [23]. Overall HPV prevalence was highest in the penile shaft (52%), followed by scrotum (40%), glans/corona (32%), urine (10%), and semen (6%). The prevalence of any HPV infection in the glans/corona was significantly higher in uncircumcised men (46%) than in circumcised men (29%) (OR 1.96; 95% CI = 1.02-3.75). Uncircumcised men also had an increased risk of oncogenic HPV infection (adjusted OR 2.51; 95% CI = 1.11-5.69) and infection with multiple HPV types in the glans/corona (adjusted OR 3.56; 95% CI = 1.50-8.50) [23].

Nicolau evaluated the prevalence of HPV DNA in 50 male partners of HPV-infected women [24]. The brushings were HPV DNA positive in 35 (70%) of the men: 32% in the high-risk HPV group, 14% in the low-risk HPV group, and 24% in both groups. HPV detection per anatomic site was 24% in the glans, 44% in the prepuce internal surface, 30% in the distal urethra, 24% in the prepuce external surface, 12% in the scrotum, and 8% in the anus. Carestiato evaluated the prevalence of human papillomavirus infection determined by hybrid capture assay in 1,481 men [25]. The hybrid capture test (HCA II) is a non-radioactive, hybridization assay, designed to detect 18 HPV types divided into high and low-risk groups. The prevalence was 9.1% in the low-risk group, 9.7% in the high risk group and 7.4% with mixed infections, giving a total prevalence of 26.2%.

Castellsagué et al. [26] has shown a lower prevalence of penile HPV in men who have been circumcised (OR = 0.37; 95% CI, 0.16-0.85). In this large multination study, Castellsagué et al. found HPV in 19.6% of 847 uncircumcised men, but only 5.5% of 292 circumcised men. After adjustment for confounding variables, circumcision remained associated with less frequent HPV infection (OR 0.37). In healthy Mexican military men HPV prevalence was 44.6%, and OR for persistent HPV was 10 times higher in uncircumcised [27]. Nielson et al. [28] examined the association between HPV infections and circumcision at the glans penis/coronal sulcus, penile shaft, and scrotum in addition to the urethra, semen, perianal area, and anal canal in 463 men. Seventy-four men (16%) were uncircumcised. Adjusted odd ratios

(AORs) for any HPV genotype and circumcision were 0.53 (95% CI = 0.28-0.99) for any anatomic site/specimen, 0.17 (95% CI = 0.05-0.56) for the urethra, 0.44 (95% CI = 0.23-0.82) for the glans/corona, and 0.53 (95% CI = 0.28-0.99) for the penile shaft. These results suggest that circumcision may be protective against HPV infection of the urethra, glans/corona, and penile shaft. Auvert et al. analyzed the effect of male circumcision (MC) on the prevalence of HR-HPV [29]. In this study, 3274 uncircumcised men were recruited, randomized into 2 groups, and followed up. MC was offered immediately after randomization to the intervention group and after the end of the follow-up period to control group participants. Urethral swab sample was collected at the 21-month visit in 1264 participants, reported by randomization group. The urethra was chosen because the detection of HPV in this anatomical site is probably not affected by circumcision status. HR-HPV prevalence was 14.8% in the intervention group and 22.3% in the control group (prevalence rate ratio [PRR] = 0.66; 95% CI = 0.51-0.86; p < .002). The percentage of each of the 13 HR-HPV genotypes was lower in the intervention group than in the control group. The prevalence of multiple HR-HPV types was lower in the intervention group than in the control group (4.2% *vs*. 9.9%; PRR = 0.43; 95% CI = 0.28-0.66; p < .001). This controlled trial showed a reduction in the risk of HR-HPV infection among men after MC.

There is an association between the mean number of female sexual partners in the year preceding the study and the presence of HPV DNA. The higher the number of sexual partners the greater, the chance of acquiring and transmitting HPV. Castellsagué et al. [26] studied uncircumcised men who had had less than five sexual partners up to the time of the study and found that 12.5% of them were positive for HPV DNA, while among men who had had more than five sexual partners up to the time of the study the percentage of HPV DNA-positive subjects increased to 44.7%. Fransceschi et al. [30] found a highly significant association (p < 0.01) between the presence of HPV DNA and the number of sexual partners up to the date of the study, with 21.1% of men having less than 10 sexual partners being positive for HPV DNA, as opposed to 43.3% of men having more than 10 sexual partners. In another study by Rombaldi et al. [31], demonstrated that the greatest risk factor (p = 0.038) for acquiring HPV DNA was related to the total number of sexual partners up to the date of the survey, with men who had the highest number of sexual partners have the highest risk (p = 0.038) of being positive for HPV DNA.

The prevalence of HPV DNA in penile carcinomas ranges between 15% and 81% (Table I). Rubin et al. evaluated the prevalence of HPV DNA in different histological subtypes of penile carcinoma, dysplasia, and condyloma [32]. HPV DNA was detected in 42% cases of penile carcinoma, 90% cases of dysplasia, and 100% cases of condyloma. In this study, although keratinizing squamous cell carcinoma (SCC) and verrucous carcinoma were positive for HPV DNA in only 34.9% and 33.3% of cases, respectively, HPV DNA was detected in 80% of basaloid and 100% of warty tumor subtypes [32]. Cubilla et al. [33] reported detection of HPV 16 in 9 of 11 (81%) cases of basaloid and 3 of 5 (60%) cases of warty SCC of the penis.

Penile cancer, like cervical cancer, is caused by high-risk HPV, but penile cancer is 10 times less common than cervical cancer [34]. Many studies have shown the presence of HPV types 16 and 18 in penile carcinoma. In a case-control study in Uganda [35] the seropositivity to HPV-16, HPV-18, or HPV-45, the most common oncogenic types of HPV, was 46% among penile cancer cases and 12% among controls (OR 5.0, 95% CI = 1.4-17.2). In another case-control study done in the United States [36], positive HPV-16 serology was found among 24% of penile cancer cases and 12% of controls (OR 1.9, 95% CI = 1.2-3.2); 80% of penile cancer tissue specimens were positive for HPV-DNA. Heideman performed molecular and serologic analyses of HPV types on a series of 83 penile cancer squamous cell carcinomas (SCCs), and compared serological findings to those of age-matched male controls (n = 83) [37]. HPV DNA of mucosal and/or cutaneous types was found in 46 of 83 (55%) penile SCCs. HPV-16 was the predominant type, appearing in 24 (52%) of 46 of penile SCCs. The majority of HPV 16 DNA-positive SCCs (18 of 24; 75%) demonstrated E6 transcriptional activity and a high viral load. HPV 16 molecular findings were strongly associated with HPV 16 L1-, E6-, and E7-antibody. Furthermore, serologic case-control analyses demonstrated that, in addition to the association of HPV 16 with penile SCC, seropositivity against any HPV type was significantly more common in patients compared with in controls. Madsen et al. examined tissue samples of 37 penile SCC patients for the presence of HPV-DNA by PCR [38]. Twenty-four (65%) were hrHPV positive, and 1 (3%) was positive to a low-risk HPV type (HPV6). By far, the predominant HPV type was HPV16, which was detected in 22 (59.5%) of the 37 examined tumors, corresponding to 92% of the 24 hrHPV-positive tumors.

Guerrero et al. [39] detected HPV DNA by polymerase chain reaction in 4 of 10 patients (40%) with penile cancer, of which HPV 18 was present in 3 patients (75%), and HPV 16 and 18 in 1 (25%). In an examination of 30 specimens of penile cancer by polymerase chain reaction and in situ hybridization assays from 23 patients, the HPV-16 genome was found in 15 patients (65%), HPV-30 in 3 (13%), and HPV-6 or HPV-11 in 2 (9%) [40]. Bezerra et al. detected HPV DNA in 30.5% (25 of 82) samples of penile carcinoma in Sao Paulo, Brazil. HPV-16 was the most frequent type detected (13 of 25, 52%). Maden et al. [42] reported that, among 67 men with penile cancer who had tumor tissues available for HPV DNA testing, 49% were positive; the majority (69.7%) of which were type 16. Rubin et al. [32] found that the most common viral type identified in penile cancer was HPV 16, which was detected in 60% of HPV positive cancers. Pascual et al. [43] studied 49 patients with penile carcinoma. Thirty-eight patients of the 49 cases were positive for HPV (77.5%). HPV 16 appeared in 32 (84.2%) of the 38 positive cases and HPV 18 in 4 (10.5%). Lont et al. [44] detected high-risk HPV DNA in 29% of the tumors, with HPV 16 being the predominant type, accounting for 76% of high-risk HPV containing SCC. Scheiner et al. [45] evaluated the presence of HPV in penile cancer in Rio de Janeiro, Brazil. HPV DNA was detected in 72% of patients with invasive carcinomas and in 50% of patients with verrucous carcinomas. High risk HPV's were detected in 15 of 54 (27.5%) patients with HPV positive invasive tumors and in 1 of 4 (25%) patients with HPV positive verrucous tumors. The HPV 16 type was observed in 12 of 23 (52%) cases. Tornesello et al. [46] evaluated HPV genotype in 41 penile cancer biopsies from Italian patients. Among the 19 HPV-positive cases (46.3%) 2 viral genotypes were identified (HPV 16 and 18) with HPV 16 accounting for 94.7% (18 out of 19) of the infections. In this study, HPV-positive patients were significantly older (65.4 ± 7.3 *vs.* 58.1 ± 14.3); all patients under 50 years were HPV negative. Senba studied the relation between penile cancer and the prevalence of HPV genotypes in northern Thailand in 88 specimens of penile tissue [47]. In this study, an in situ hybridization (ISH) method was used to detect and localize HPV-DNA. Sensitive HPV polymerase chain reaction (PCR) procedure was used to detect and localize HPV-DNA, and DNA sequencing was used to identify the HPV genotype. HPV-DNA was detected in 53.8% and 81.5% of cases of penile cancer, using ISH and PCR, respectively. The most prevalent genotype was the high-risk HPV-18, found in 55.4% of the cases (as single infection in 32.3% and as multiple infections in 23.1%), followed by the low-risk HPV-6 found in 43.1% of the cases (as single infection in 24.6% and as multiple infections in 18.5%).

Table I. Prevalence of HPV-DNA in penile carcinomas.

REFERENCE	NO.	HPV-POSITIVE %
McCance [36]	53	51
Madsen [38]	37	68
Iwasawa [48]	111	63
Maden [42]	67	49
Chan [49]	41	15
Cupp [50]	42	55
Gregoire [51]	117	22
Picconi [52]	38	71
Rubin [32]	142	42
Guerrero [39]	10	40
Tornesello [46]	41	46
Scheiner [45]	80	72
Bezerra [41]	82	30
Pascual [43]	49	77
Giuliano [21]	303	65
Nielson [22]	463	65
Rombaldi [31]	99	54
Qiang [53]	28	61
Suzuki [54]	13	54
Senba [47]	65	81
Salazar [55]	54	65

Finally, Merck has announced recently that Gardasil, the vaccine that protects women from common strains of the human papillomavirus, has a 90% efficacy in preventing external genital lesions caused by HPV types 6, 11, 16 and 18 in men aged 16-26 years [48], suggesting that this vaccine may be efficacious in preventing infection and lesions from HPV in men. Future studies are warranted.

In conclusion, the incidence of penile cancer is low in developed countries, contrary to underdeveloped nations where the incidence could be as high as 3.5 per 100,000 inhabitants. The evidence suggests that circumcision may be protective against HPV infection of the urethra, glans/corona, and penile shaft. Similar to women, there is a direct proportional relationship between the number of sexual partners and the presence of HPV-DNA, and hence, a higher risk for developing a malignant pathology. In men with penile cancer, the prevalence of HPV infection ranges between 15 and 81%, and the most common oncogenic HPV genotypes found are 16 and 18.