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Original Article

HPV frequency in penile carcinoma of Mexican patients: important contribution of HPV16 European variant

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Abstract: The role of human papillomavirus (HPV) infection in penile carcinoma (PeC) is currently reported and about half of the PeC is associated with HPV16 and 18. We used a PCR-based strategy by using HPV general primers to analyze 86 penile carcinomas paraffin-embedded tissues. Some clinical data, the histological subtype, growth pattern, and differentiation degree were also collected. The amplified fragments were then sequenced to confirm the HPV type and for HPV16/18 variants. DNA samples were also subjected to relative real time PCR for hTERC gene copy number. Some clinical data were also collected. Global HPV frequency was 77.9%. Relative contributions was for HPV16 (85%), 31 (4.4%), 11 (4.4%), 58, 33, 18, and 59 (1.4% each one). Sequence analysis of HPV16 identified European variants and Asian-American (AAb-c) variants in 92% and in 8% of the samples, respectively. Furthermore hTERC gene amplification was observed in only 17% of the cases. Our results suggest that some members of HPV A9 group (represented by HPV16, 58, and 31) are the most frequent among PeC patients studied with an important contribution from HPV16 European variant. The hTERC gene amplification could be poorly related to penile epithelial tissue.

Keywords: HPV, penile carcinoma, México

Introduction

Squamous cell carcinoma (SCC) of the penis is a relatively rare disease in developed countries, where age-standardized incident rates range < 1/100,000 men, while, in South America (Brazil and Columbia) and Africa (Uganda), the incident can reach 4/100,000 men. In Europe, about 4,000 cases are diagnosed each year, which comprises less than 0.5% of all cancers [1]. Less than 0.5% is reported for Mexico [2]. The disease generally occurs late in life, and an age-related incidence that rises continuously to reach its highest level after 70 years of age.

Epidemiologic and molecular studies have suggested a functional role of high-risk human papillomavirus (hr-HPV) for a subset of penile SCC (PSCC) [3-9]. In a comprehensive report of more than 30 major penile cancer studies (published from 1986-June 2008) evaluating the HPV prevalence among the different histological types was carried out. It was observed that about half of the penile tumors were associated with HPV16 and 18 with a little presence of other genotypes [10]. On the other hand, several reports had shown that HPV is not the only risk factor for SCC development, suggesting that additional cellular factors are needed for the progression of the disease [7, 8]. For instance,

the gain of chromosome 3q (probably hTERC gene) that occurs in HPV16-infected, aneuploid cells represents a pivotal genetic aberration at the transition from severe dysplasia/CIS to invasive squamous cervical carcinoma (CC) [11]. Thus, it has been report and suggested to hTERC as potential marker in cancer [12]. At present there is not any report about of this genetic event in PSCC.

To contribute with penile cancer research and HPV infection worldwide data, as some other genetic alteration related to SCC, in the present work a group of fixed and paraffin embedded specimens from Mexican patients affected by penile cancer were analyzed by PCR-based approach for the presence of HPV sequences, and by real-time PCR looking for *hTERC* gene copy gain.

Material and methods

Tissue samples

We examined 86 formalin-fixed paraffin-embedded (FFPE) penile cancer specimens. These samples were collected (in the period 1988 to 2004) from two Hospitals, at Department of Pathology, Hospital de Oncología, CMNSXXI-IMSS, and Hospital General Hospital de Mexico, SS, in Mexico City. The described procedures have been approved by the local committee (CLICHO) at IMSS institutional board. The histopathological subtypes consisted of 52 cases of typical squamous, 1 sarcomatoid, 4 condilomatous, 2 papillary, 2 verrucous, 10 carcinoma in situ, and 15 mixed carcinomas of the penis. According to archives, all the patients were subjected to penectomy and lymphadenectomy; the cases were T1-2, N1-2, M0 stages (TNM classification). At least in a four years period of follow-up they did not present any recurrence of the disease or death; unfortunately at this time all the patients abandoned the physician routine assistance.

A tissue section of each specimen was hematoxilin and eosin stained and blindly analyzed by two independent pathologists to confirm the diagnosis. Growth pattern (superficially spreading, vertical growth, verruciform, mixed, multicentric), degree of differentiation (well, moderately, and poorly differentiated), and koilocytic atypia, were also evaluated. Clinical variables such as age, sexually transmitted diseases and the number of sexual partners were collected; unfortunately in some cases the information was not available. All cases were uncircumcised.

DNA extraction

Two 15 μm non-stained tissue sections were mounted on clean slides, deparaffinized and rehydrated by standard methods. To avoid false negatives, defined tumor areas were manually microdissected under the light microscope (20x objective) with an sterilized needle, the scrapped tissue was then collected in a microtube containing 500 μl of digestion buffer (100 mM NaCl, 10 mM Tris-HCl pH 8, 25 mM EDTA pH 8, 0.5% SDS, and 0.1 mg/ml Proteinase K), and incubated at 55 °C for 48 hours. DNA was extracted by means of Wizard Extraction kit (Promega, Madison WI, USA). HPV DNA was detected by using two pairs of oligonucleotides the consensus primers Gp5+/6+ (150 bp) [12], or HPV16/E6 specific oligonucleotides (126 bp) [13]. The PCR solution contained: 200 ng of tumor DNA, 1x buffer (50 mM KCl, 10 mM Tris-HCl, 0.1% Triton X-100), 2 mM $MgCl_2$, 0.2 mM of each dNTP, 50 pmol of each primer and 2 U of Taq DNA polymerase (Promega Co.) in a 50 μl of final volume. Thirty-five amplification cycles employing a thermal cycler (MJ Research Minicycler) were carried out for Gp5+/6+ and HPV16/E6 primers with the following program: denaturation at 94 °C for 30 sec, annealing at 45 °C for 1.5 min, and extension at 72 °C for 1.5 min, with a final extension at 72 °C for 7 min [13, 14]. For each series of five samples, SiHa cell line DNA (HPV16+) as positive control was used, C33 cell line (HPV-) and lymphocyte DNA were included as negative controls.

Prior to demonstrate HPV sequences in the FFPE DNA samples, the DNA quality for amplification reaction was demonstrated when the samples were subjected to PCR by using *RET* gene primers (160 bp fragment). In this context, DNA were analyzed for the presence of HPV16/E6 primers, and those negatives for this pair of primers, then were subjected to a second PCR by using GPs primers that they can recognize a wide range of HPV types. The amplified products can recognize a wide range of HPV types. The amplified products were then sequenced by using the Wizard SV Gel and PCR-Clean-up System Kit (Promega Co.). The amplified products were then sequenced by

Table 1. HPV frequency in penile carcinomas of Mexican patients

Tumor Histology	n	HPV groups							
		A9				A7		A10	Neg.
		16	31	33	58	18	59	11	
Typical*	52	45	0	0	0	0	0	0	7
Mixed	15	2	2	0	0	1	0	1	9
Carcinoma *in situ*	10	10	0	0	0	0	0	0	0
Condilomatous	4	0	0	1	0	0	0	1	2
Papillary	2	0	0	0	0	0	0	1	1
Verrucous	2	0	1	0	1	0	0	0	0
Sarcomatoid	1	0	0	0	0	0	1	0	0
TOTAL	86	57	3	1	1	1	1	3	19

*non-keratinizing; n=total of cases.

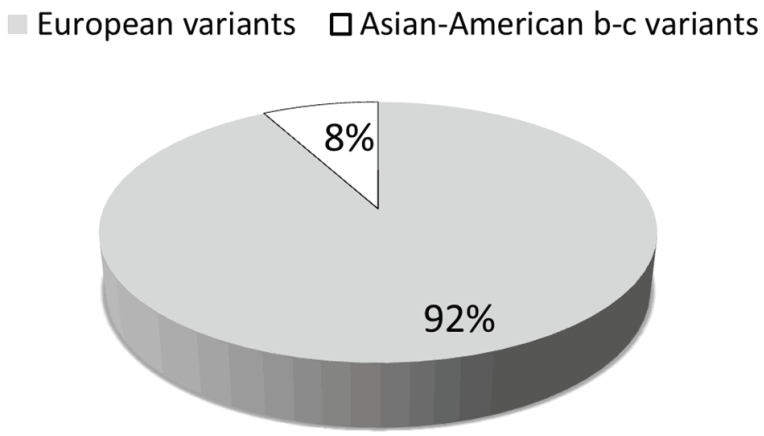

Figure 1. HPV16 variants in Mexican penile cancer samples.

using an Applied Biosystems 373 automated DNA sequencer (Perkin Elmer, Applied Biosystems), and the obtained sequences were aligned and compared with existing databases using the BLAST program via Internet.

hTERC gene copy number

Thirty four samples: 26 HPV positives and 8 HPV negatives were analyzed by semi- quantitative real time PCR. hTERC D-LUX probe labeled with FAM molecules designed with designer (Invitrogen.com) were used for these analyses (FO 5' cggttttaaggtagtcgaggtgaaccg; RO 5' tgcgtctttacttccgaccttc). Fifty ng of DNA of each sample was subjected to real time PCR in a 10 µl final volume contained: 5 µl QPCR Supermix UDG (Invitrogen Co., Carlsbad, CA, USA), 0.3 U of Taq Polymerase platinum. The amplification conditions were are followed: enzyme activation 2 min 50 °C, and 2 min to 94 °C, 45 cycles of 10 sec 94 °C, 5 sec at 62 °C and 10 sec at 72 °C. The samples were run in a LigthCycler 4.2; and amplification reaction was considered if gene/reference > 2; in this case, as reference was used the β2m probe (FO 5' acataccttggttgatccactt; RO 5' catctgttgctctatacgtggcagatg). The relative genomic copy numbers was calculated using the comparative Ct method.

Statistical analysis

The statistical analysis was performed using the statistical software SPSS 15.0. The X^2 test was conducted and considered of statistical significance with a level of $p < 0.05$.

Results

The age of the patients ranged from 24 to 94 years (mean age at diagnosis of 58 years). The distribution by age groups was: 43% for > 65 years, 39% between 41 and 65, and 7% for < 41 years. The differentiation degree showed 60% moderately, 21% well, and 19% poorly differentiated, respectively. In contrast, koilocytic atypia was present in 73% of the tumors.

In order to know the present HPV infection, all DNA samples were subjects to PCR analysis to detect HPV sequences. The PCR results allowed us to determine the overall HPV frequency in 77.9% (67/86) of the cases. HPV presence and histological types are showed in the **Table 1**.

HPV16 was the most frequent type among all HPV samples (85%) followed by HPV types 31 and 11 (4.4% each one), whereas HPV18, 33, 58 and 59 were present in one sample each (1.4%). In only five cases (7.4%) multiple infection of HPV was detected, three cases for HPV16/31 and two cases for HPV16/33. Most of the patients between 54 to 73 years presented HPV16 sequences, while HPV31, 11 and 18 were spread among ages.

Table 2. Correlation between *hTERC* gene amplification and HPV status in Mexican penile cancer samples

		hTERC (# gene copies)		
	n	2X	> 2X	*P* value*
HPV status	34	28	6	
positive	26	20	6	0.134
negative	8	8	0	

*X^2-test; 2X=two copies of the gene.

HPV16 positive samples were then sequenced to identify the variants. The results show that HPV16 European prototype (E) was the most frequently found (65%, 37/57), followed by the European C188T subclass (27%); the remaining samples (8%) were Asian-American variants b-c (AAb-c). Thus, HPV16 European variant was present in 92% of the Mexican penile cancer samples (**Figure 1**). Interestingly, only in two samples HPV16+, sequence variation to E6 gene at nucleotide G153C (coding for Arg à Thr at position 24), or nucleotide G204A (Lys à Arg at position 41). These changes have not been previously described. For HPV18+ samples harbored the prototype sequences. These data could suggest an extremely low rate of genomic changes.

After that the samples were analyzed by HPV sequences, only 34 DNA samples (HPV+ and HPV-) were available for hTERC analysis. The fold change in hTERC gene of some tumor samples was measured in comparison to β2m gene reference. The *hTERC* showed gain of copy number in 6 samples (17%); in this context, we did not observed any relationship between HPV status and *hTERC* gene gain copy number (**Table 2**).

Discussion

The PSCC and its relationship with HPV have been reported [15-18]. A systematic review in almost 1500 cases of HPV prevalence in this kind of cancer has been informed: North America (48.7%), South America (39.7%), Europe (45.9%), and Asia (59.3%), being the three most common HPV types: HPV16 (30.8%), HPV6 (6.7%), and HPV18 (6.6%) [19]. Our data could show some differences respect to worldwide report.

Recently, it has been reported the most frequent HPV types in cervical cancer (CC) in Mexico (data submitted to publish, Salcedo *et al*) [20]. Interestingly, most of the HPV types found in CC are quite similar found in the present work, HPV16, 18, 31, 33, 58 and 59. These data could support a sexual transmission of HPV in a group of penile cancer.

A wide variety of HPV types is present in CC; and most of them are also associated with penile cancer, predominantly HPV16, 18 with a little presence of other genotypes [10, 21].

As expected, HPV16 was the most frequently found in the present study, followed by 31, 11, 18/33/58/59. Our data support previous results of HPV types in PSCC [10]. This could suggest that these viral types are commonly associated to penis tissue.

In this situation, HPV types belonging to the A9 species were the most common HPV types present in the penile lesions (92%), while viral types HPV18, and 33 (A7 subgroup) were less frequently found. Similar results were found in CC [22]. These results suggest that viral types present in Mexican PSCC are phylogenetically related. It is important to note a low HPV18 presence in our data, as happen in CC [22], and this datum could suggests that this viral type distribution might vary depending on geographic region. We did not discard the role of the genetic background of our population.

Interestingly, HPV31 was detected as second place of frequency in our population. This finding supports previous data [22]. We can to consider that additional studies should be performed to explain the probable differential biologic behavior of HPV31 and HPV16 in the penile lesions. Also HPV11 was found only in 4 samples, but the association between low-risk HPVs and malignant lesions has been found in other malignancies [23]. However, we did not discard multiple-infection with an hr-HPV type present in an extremely low DNA copies. This fact could be supported by recent reports in healthy men where the authors report in 50% the presence of multiple HPV infections [24]. According with our present data, we could hypothesize that multiple–HPV infection in penile cancer could be a poor contribution.

It has been proposed that the distinct HPV variants correlate with the ethnicity of population's rather than geographical distribution [25]. The high frequency of HPV16 European variant

found in the present study is similar to frequencies of this virus type for CC [26, 27]. Thus, our data could support that HPV variants could correlate with the ethnicity. Interestingly, a recent report about HPV variants in penile cancer at Italy showed an increased frequency of HPV16 non-European variant [28]. This discrepancy should implicate the need for evaluating the risk of progression associated with each variant in a larger study population.

On the other hand, circumcision and its relation to penile cancer and HPV infection has been a subject of intense debate [29-32], indicating that male circumcision is associated with a reduced risk of penile HPV infection. In our study, all the patients were uncircumcised. This is an important factor to be considered in nations where circumcision is not a common practice. Thus, our data could support the important role of circumcision to prevent HPV infection and penile cancer development.

It is often difficult to draw a distinction between association and causation in cancer [33, 34]. Due to small number of the samples, some data as differentiation degree, histological type, clinical stage and HPV types other than HPV16 (and 18) in the pathogenesis of penile cancer should not be concluded, and also studies examining de HPV expression should be carried out. In addition, in order to robust and evaluate differential survival role to HPV, this report could also serve to call for a large international collaborative study to evaluate all these aspects.

Based in the present data, available prophylactic HPV vaccination could prevent the development of slightly more than half of penile cancer in the Mexican population. These results might be considered for earlier HPV screening in Mexican men; the new development of HPV vaccine design harboring another HPV types by example 31, 33, 52 and 58, and the subsequent clinical course are needed. This data support the recent report about the nine-valent vaccine against HPV [35]. Although the rarity in frequency of this type of cancer, a limitation of the present study is the sample size, with the consequent limited analysis of some clinical variables.

Respect to hTERC gene alteration in PSCCs samples, the low percentage of hTERC amplification in PSCC suggests that hTERC gain copy number is not associated to HPV infection in PSCC samples. Further, the hTERC amplification could be related to cellular event in the transformed cell.

In summary, our results are showing: 1) the HPV A9 group is the most frequent in penile cancer of Mexican population; 2) an important contribution of HPV16 European variant in our population with this tumor, and 3) hTERC amplification could be a rare genetic event in PSCC and also not related to HPV infection.

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Disclosure of conflict of interest

We have no conflict of interest in association with this work.

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References

[1] Parkin DM, Whelan SL, Ferlay J. Cancer Incidence in Five Continents. Lyon, France: IARC Scientific Publication 2002, pp: 155.

[2] Registro histopatológico de neoplasias en México. México, DF: Secretaría de Salud, 2001. www.dgepi.salud.gob.mx.

[3] Gross G, Pfister H. Role of human papillomavirus in penile cancer, penile intraepithelial squamous cell neoplasias and in genital warts. Med Microbiol Immunol 2004 Feb; 193: 35-44.

[4] Van Doornum GJ, Korse CM, Buning-Kager JC, Bonfrer JM, Horenblas S, Taal BG, Dillner J. Reactivity to human papillomavirus type 16 L1 virus-like particles in sera from patients with genital cancer and patients with carcinomas at five different extragenital sites. Br J Cancer 2003 Apr 7; 88: 1095-100.

[5] Carter JJ, Madeleine MM, Shera K, Schwartz SM, Cushing-Haugen KL, Wipf GC, Porter P, Daling JR, McDougall JK, Galloway DA. Human papillomavirus 16 and 18 L1 serology com-

pared across anogenital cancer sites. Cancer Res 2001 Mar 1; 61: 1934-40.

[6] Bjørge T, Dillner J, Anttila T, Engeland A, Hakulinen T, Jellum E, Lehtinen M, Luostarinen T, Paavonen J, Pukkala E, Sapp M, Schiller J, Youngman L, Thoresen S. Prospective seroepidemiological study of role of human papillomavirus in non-cervical anogenital cancers. BMJ 1997 Sep 13; 315: 646-9.

[7] Ferreux E, Lont AP, Horenblas S, Gallee MP, Raaphorst FM, von Knebel Doeberitz M, Meijer CJ, Snijders PJ. Evidence for at least three alternative mechanisms targeting the p16INK4A/cyclin D/Rb pathway in penile carcinoma, one of which is mediated by high-risk human papillomavirus. J Pathol 2003 Sep; 201: 109-18.

[8] Rubin MA, Kleter B, Zhou M, Ayala G, Cubilla AL, Quint WG, Pirog EC. Detection and typing of human papillomavirus DNA in penile carcinoma: Evidence for multiple independent pathways of penile carcinogenesis. Am J Pathol 2001 Oct; 159: 1211-8.

[9] Levi JE, Rahal P, Sarkis AS, Villa L. Human papillomavirus DNA and p53 status in penile carcinomas. Int J Cancer 1998 Jun 10; 76: 779-83.

[10] Miralles-Guri C, Bruni L, Cubilla AL, Castellsagué X, Bosch FX, de Sanjosé S. Human papillomavirus prevalence and type distribution in penile carcinoma. J Clin Pathol 2009 Oct; 62: 870-8.

[11] Heselmeyer K, Schröck E, du Manoir S, Blegen H, Shah K, Steinbeck R, Auer G, Ried T. Gain of chromosome 3q defines the transition from severe dysplasia to invasive carcinoma of the uterine cervix. Proc Natl Acad Sci U S A 1996 Jan 9; 93: 479-84.

[12] He C, Xu C, Xu M, Yuan Y, Sun Y, Zhao H, Zhang X. Genomic amplification of hTERC in paraffin-embedded tissues of cervical intraepithelial neoplasia and invasive cancer. Int J Gynecol Pathol 2012 May; 31: 280-5.

[13] Manos MM, Ting Y, Wright DK. Use of Polymerase Chain Reaction amplification for the detection of genital Human Papillomaviruses. In Furth M & Greaves M (eds.). Cancer cells 7 / Molecular Diagnostics of Human Cancer. U.S.A.: Cold Spring Harbor Lab Press. 1989, pp: 209-214.

[14] de Roda Husman AM, Walboomers JM, van den Brule AJ, Meijer CJ, Snijders PJ. The use of general primers GP5 and GP6 elongated at their 39 ends with adjacent highly conserved sequences improves human papillomavirus detection by PCR. J Gen Virol 1995 Apr; 76: 1057-62.

[15] Dillner J, von Krogh G, Horenblas S, Meijer CJ. Etiology of squamous cell carcinoma of the penis. Scand J Urol Nephrol Suppl 2000; 189-93.

[16] Rubin MA, Kleter B, Zhou M, Ayala G, Cubilla AL, Quint WG, Pirog EC. Detection and typing of human papillomavirus DNA in penile carcinoma: Evidence for multiple independent pathways of penile carcinogenesis. Am J Pathol 2001 Oct; 159: 1211-8.

[17] Cubilla AL, Velazques EF, Reuter VE, Oliva E, Mihm MC Jr, Young RH. Warty (condylomatous) squamous cell carcinoma of the penis: A report of 11 cases and proposed classification of 'verruciform' penile tumors. Am J Surg Pathol 2000 Apr; 24: 505-12.

[18] Cubilla AL, Reuter VE, Gregoire L, Ayala G, Ocampos S, Lancaster WD, Fair W. Basaloid squamous cell carcinoma: A distinctive human papilloma virus-related penile neoplasm—A report of 20 cases. Am J Surg Pathol 1998 Jun; 22: 755-61.

[19] Backes DM, Kurman RJ, Pimenta JM, Smith JS. Systematic review of human papillomavirus prevalence in invasive penile cancer. Cancer Causes Control 2009 May; 20: 449-57.

[20] Peralta-Rodríguez R, Romero-Morelos P, Villegas-Ruíz V, Mendoza-Rodríguez M, Taniguchi-Ponciano K, González-Yebra B, Marrero-Rodríguez D, Salcedo M. Prevalence of human papillomavirus in the cervical epithelium of Mexican women: meta-analysis. Infect Agent Cancer 2012; 7: 34.

[21] Heideman D, Waterboer T, Pawlita M, Diemen PD, Nindl I, Leijte J, Bonfrer JMG, Horenblas S, Meijer CJ, Snijders P. Human Papillomavirus-16 Is the Predominant Type Etiologically Involved in Penile Squamous Cell Carcinoma. J Clin Oncol 2007 Oct 10; 25: 4550-6.

[22] Piña-Sánchez P, Hernández-Hernández D, López-Romero R, Vázquez-Ortíz G, Pérez-Plasencia C, Lizano-Soberón M, González-Sánchez JL, Cruz-Talonia F, Salcedo M. Human papillomavrius–specific viral types are common in Mexican women affected by cervical lesions. Int J Gynecol Cancer 2006 May-Jun; 16: 1041-7.

[23] Syrjänen S. The role of human papillomavirus infection in head and neck cancers. Ann Oncol 2010 Oct; 21 Suppl 7: vii243-5.

[24] Lajous M, Mueller N, Cruz-Valdéz A, Aguilar LV, Franceschi S, Hernández-Avila M, Lazcano-Ponce E. Determinants of prevalence, acquisition, and persistence of human papillomavirus in healthy Mexican military men. Cancer Epidemiol Biomarkers Prev 2005 Jul; 14: 1710-6.

[25] Chan SY, Ho L, Ong CK, Chow V, Drescher B, Durst M, ter Meulen J, Villa L, Luande J, Mgaya HN, Bernard H. Molecular variants of human papillomavirus type 16 from four continents suggest ancient pandemic spread of the virus and its coevolution with humankind. J Virol 2002 Apr; 66: 2057-2066.

[26] Calleja-Macias IE, Kalantari M, Huh J, Ortiz-Lopez R, Rojas-Martinez A, Gonzalez-Guerrero JF, Williamson AL, Hagmar B, Wiley DJ, Villarreal L, Bernard HU, Barrera-Saldaña HA. Genomic diversity of human papillomavirus-16, 18, 31, and 35 isolates in a Mexican population and relationship to European, African, and Native American variants. Virology 2004 Feb 20; 319: 315-23.

[27] Lizano M, De la Cruz-Hernández E, Carrillo-García A, García-Carrancá A, Ponce de Leon-Rosales S, Dueñas-González A, Hernández-Hernández DM, Mohar A. Distribution of HPV16 and 18 intratypic variants in normal cytology, intraepithelial lesions, and cervical cancer in a Mexican population. Gynecol Oncol 2006 Aug; 102: 230-5.

[28] Tornesello ML, Duraturo ML, Losito S, Botti G, Pilotti S, Stefanon B, De Palo G, Gallo A, Buonaguro L, Buonaguro FM. Human papillomavirus genotypes and HPV16 variants in penile carcinoma. Int J Cancer 2008 Jan 1; 122: 132-37.

[29] Daling JR, Madeleine MM, Johnson LG, Schwartz SM, Shera KA, Wurscher MA, Carter JJ, Porter PL, Galloway DA, McDougall JK, Krieger JN. Penile cancer: Importance of circumcision, human papillomavirus and smoking in in situ and invasive disease. Int J Cancer 2005 Sep 10; 116: 606-16.

[30] Lont AP, Kroon BK, Horenblas S, Gallee MP, Berkhof J, Meijer CJ, Snijders PJ. Presence of high-risk human papillomavirus DNA in penile carcinoma predicts favorable outcome in survival. Int J Cancer 2006 Sep 1; 119: 1078-81.

[31] Houle AM. Circumcision for all: the pro side. Can Urol Assoc J 2007 Nov; 1: 398-400.

[32] Castellsagué X, Bosch FX, Muñoz N, Meijer CJ, Shah KV, de Sanjose S, Eluf-Neto J, Ngelangel CA, Chichareon S, Smith JS, Herrero R, Moreno V, Franceschi S; International Agency for Research on Cancer Multicenter Cervical Cancer Study Group. Male circumcision, penile human papillomavirus infection, and cervical cancer in female partners. N Engl J Med 2002 Apr 11; 346: 1105-12.

[33] Pagano JS, Blaser M, Buendia MA, Damania B, Khalili K, Raab-Traub N, Roizman B. Infectious agents and cancer: criteria for a causal relation. Semin Cancer Biol 2004 Dec; 14: 453-71.

[34] Brower V. Accidental passengers or perpetrators? Current virus-cancer research. J Natl Cancer Inst 2004 Feb 18; 96: 257-58.

[35] Serrano B, Alemany L, Tous S, Bruni L, Clifford GM, Weiss T, Bosch FX, de Sanjosé S. Potential impact of a nine-valent vaccine in human papillomavirus related cervical disease. Infect Agent Cancer 2012 Dec 29; 7: 38.