Human papillomavirus detection through polymerase chain reaction in penile cancer patients: experience at the Urology Department of the Hospital General “Dr. Manuel Gea González”


ABSTRACT

Introduction: Carcinoma of the penis is a rare tumor and little is known about the role human papillomavirus plays in this type of cancer. In the last few years this virus has been recognized as a possible etiologic agent in penile cancer. Cytologic changes suggestive of human papillomavirus infection are similar to those observed in women (koilocytosis, dyskeratocytosis, and nuclear changes). The mechanism by which human papillomavirus makes a malignant transformation is probably through the E6 and E7 viral genes. These form products of the p53 and pRb suppressor genes, resulting in uncontrolled cell proliferation. Basaloid and verrucous histological subtypes are the most frequently related. A 30.5% prevalence has been shown in paraffin-embedded samples using polymerase chain reaction.

RESUMEN

Introducción: El carcinoma de pene es una neoplasia poco frecuente. Se sabe poco acerca del papel que juega el virus del papiloma humano (VPH), en este tipo de neoplasia. En los últimos años, este virus ha sido reconocido como posible agente etiológico en el cáncer de pene. Los cambios citológicos que sugieren infección por VPH, son similares a los observados en mujeres (coilocitosis, disqueratitis y cambios nucleares). El mecanismo por el cual el VPH conduce a la transformación maligna, es probable que sea mediada a través de dos genes virales (E6 y E7). Estos forman productos del gen supresor p53 y pRb, lo que produce un crecimiento celular descontrolado. Los subtipos histológicos relacionados con mayor frecuencia son el basaloid e verrucoso. Se ha demostrado una prevalencia del 30.5%, en muestras fijadas en parafina, usando reacción en cadena de polimerasa (PCR).
Objective: To evaluate the presence of human papillomavirus through the polymerase chain reaction technique in samples from nineteen patients with invasive penile cancer that were reviewed within the time frame of 2000-2011, as well as to identify the most frequent human papillomavirus genotypes.

Methods: Within the time frame of 2000 to 2011, nineteen paraffin-embedded samples with histopathologic report of penile cancer were reviewed. Diagnosis was made according to clinical history, physical examination, and biopsy.

Results: Deoxyribonucleic acid for human papillomavirus was detected in seven of the nineteen samples studied (36.8%) and genotypes 16, 18, and 32 were identified.

Conclusions: The present series showed an association with human papillomavirus infection in 37% of the penile cancer patients studied.

Keywords: Penile cancer, human papillomavirus, polymerase chain reaction, Mexico.

INTRODUCTION

Human papillomavirus (HPV) is closely related to uterine neck cancer and its premalignant lesions. In contrast to the numerous data on HPV infection in women, little is known about this infection in men, complicating the comprehension of its epidemiology and the clinical aspects of HPV infection in this sex. Due to its elevated prevalence and high infection rate, HPV frequently spreads among sexually active persons, and men can be considered important transmitters of this infection in women. 1

HPV infection diagnosis in men is usually made through biopsy carried out only when there is a suspicious lesion or if the female partner has been diagnosed with HPV. Therefore HPV is not diagnosed as frequently in men as it is in women. 2

Penile cancer is a rare tumor with higher rates in underdeveloped countries. Little is known about the relation or role HPV has in this type of tumor. In the last few years this virus has been recognized as a possible etiologic agent in the disease. The virus can affect the squamous epithelium of the male genitals. 3

In men, cytologic changes suggesting HPV infection are similar to those observed in women (koilocytosis, dyskeratocytosis, and nuclear changes). However there is low frequency of koilocytosis (4-5% of samples) actually found in penile tissue, making HPV diagnosis through cytologic methods difficult. 4

There have even been reports of koilocytosis in 4.7% of samples of the distal urethra and 1.6% of the corona of glans penis and the internal surface of the prepuce, compared with 13.5% detected through biopsy. In addition, the small number of cells in the penile smear and their low adhesion makes cytologic analysis difficult. Schneider et al. observed that the sensitivity of cytologic HPV diagnosis in the uterine neck could be improved by applying a panel of non-classic cytologic signs: mild koilocytosis, mild dyskeratocytosis, clear cytoplasm, keratohyalin granules, filament condensation, nuclear hyperchromatism, binucleation or multinucleation, and perinuclear halos. These showed more significant statistical correlation with HPV infection. 5 When these signs combine their discriminating validity they can correctly identify 84% of positive HPV cases and 92% of negative HPV smears, thus improving cytologic sensitivity. Even though it is still controversial, liquid-based cytology showed increased cytologic sensitivity when compared with conventional cytology.

To the best of the present authors’ knowledge, there is still not much information in the literature on the accuracy of liquid-based cytology or the application of...
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Penile cancer is a relatively rare disease. It generally originates in the internal epithelium of the prepuce and the glans penis. It shares a similar pathology and natural history with squamous cell carcinoma of the oropharynx and female genitals (uterine neck, vagina, and vulva) and the anus. Phimosis, poor hygiene, and smoking are principal risk factors for penile cancer. 7

The prevalence of penile cancer varies according to geographic region and ethnic origin. The mechanism by which human papillomavirus (HPV) proceeds to malignant transformation is probably through the E6 and E7 viral genes that are actively transcribed in cells infected by HPV. E6 and E7 are joined to proteins, forming products of the p53 and pRb suppressor genes, producing uncontrolled cell proliferation. Even though HPV genes have been detected in almost 100% of uterine neck cancers, the presence of HPV infection in penile cancer is quite variable. HPV has been recognized as a possible etiologic agent in carcinoma of the penis, but its role in disease development and its correlation with prognosis is still not clear. 8,9

HPV is associated with benign and malignant lesions and high risk HPV is probably the cause of anogenital cancer. Up to the present time more than 100 HPV genotypes have been reported. Muñoz et al. carried out a review of 11 case-and-control studies from 9 countries and classified 18 high risk genotypes: 16, 18, 26, 31, 33, 35, 39, 45, 51, 53, 52, 56, 58, 59, 66, 68, 73, and 82.10,11

The prevalence of penile cancer subtypes is similar to that observed in carcinoma of the vulva. Histologic subtypes most frequently related are the basaloid and verrucous subtypes. 12

McCance et al. reported 49% HPV positivity by means of Southern blot for DNA detection in HPV. Bezerra et al. showed 30.5% prevalence in paraffin samples, using polymerase chain reaction (PCR). 13-16

The objectives of the present study were to evaluate the presence of HPV in 19 patients with invasive penile cancer that were surgically managed within the time frame of 2000-2011. HPV and its associated genotypes were identified by means of PCR.

METHODS

Nineteen paraffin-embedded samples that had histopathologic report of penile cancer were collected with the collaboration of the pathology department from patients that had undergone surgery at the Hospital "Dr. Manuel Gea González" within the time frame of 2000-2011. Diagnosis was made based on clinical history, physical examination, and biopsy. Age, clinical stage, and histology were evaluated (Table 1).

HISTOPATHOLOGIC CHARACTERISTICS:

All paraffin samples were reevaluated by the same pathologist who considered the following parameters:

1. Vertical infiltration depth, measured from the superficial layer to the deepest point.
2. Histologic grade divided into well differentiated, moderately differentiated, and poorly differentiated (Table 1).

HPV DNA SEQUENCE ANALYSIS

Primary tumors in 19 patients were analyzed in paraffin-embedded sections. The area to be evaluated was selected, extracted, and deparaffinized at a temperature of 40-50°C for 15 minutes on a sterile inclusion slide. The specimen was placed in xylene at 35°C for 15 minutes to remove the remaining paraffin from the tissue. Sample was placed in 100% absolute alcohol that removed the xylene at a temperature of 40°C. The sample was then placed in 96% alcohol at a temperature of 96°C for 15 minutes. After that, sample was placed in distilled water for 5 minutes to hydrate the tissue. Finally sample was placed in Preservcyt® in correctly labeled Eppendorf tubes in autoclave.

DNA was extracted, precipitated, and analyzed by means of polymerase chain reaction (PCR). Primers were used to amplify the L1 region of the HPV (product of 150 base pairs [bp]) and for the human beta-globin gene (product of 110 bp), which was used as reaction control.

RESULTS

From the 19 samples analyzed, HPV DNA was detected in 7 patients (36.8%) and koilocytosis was observed in 1 of the samples. HPV 16 was the most frequent genotype detected in 5 patients, 4 with histopathologic report of squamous cell carcinoma and 1 of verrucous carcinoma. Other genotypes found were genotype 18 with sarcomatoid squamous cell carcinoma histology and genotype 32 with basaloid and sarcomatoid histology (Table 1).

DISCUSSION

Human papillomavirus (HPV) is presenting as an increasingly evident etiologic factor in penile cancer. Its
prevalence in tumor tissue varies from 15-71%, while some publications report from 30.5-45%. In all studies genotype 16 is the most frequent. The variability found in the different studies is probably due to the type of population as well as to the detection method employed. The results of the present study in relation to the presence of HPV in penile cancer and the frequency of genotype 16 are similar to those found in the international medical literature.

### CONCLUSIONS

The present study showed an association with HPV infection in 37% of patients with penile cancer, making HPV a possible contributor to the development and natural history of penile cancer in this case series.

### REFERENCES

Muñoz-Ibarra EL, et al. Human papillomavirus detection through polymerase chain reaction in penile cancer patients: experience at the Urology Department of the Hospital General “Dr. Manuel Gea González”.


