



Human Papilloma Virus: Prevalence, distribution and predictive value to lymphatic metastasis in penile carcinoma

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ABSTRACT

Objectives: To evaluate the prevalence, distribution and association of HPV with histological pattern of worse prognosis of penile cancer, in order to evaluate its predictive value of inguinal metastasis, as well as evaluation of other previous reported prognostic factors.

Material and Methods: Tumor samples of 82 patients with penile carcinoma were tested in order to establish the prevalence and distribution of genotypic HPV using PCR. HPV status was correlated to histopathological factors and the presence of inguinal metastasis. The influence of several histological characteristics was also correlated to inguinal disease-free survival.

Results: Follow-up varied from 1 to 71 months (median 22 months). HPV DNA was identified in 60.9% of sample, with higher prevalence of types 11 and 6 (64% and 32%, respectively). There was no significant correlation of the histological characteristics of worse prognosis of penile cancer with HPV status. Inguinal disease-free survival in 5 years did also not show HPV status influence ($p = 0.45$). The only independent pathologic factors of inguinal metastasis were: stage $T \geq T1b-T4$ ($p = 0.02$), lymphovascular invasion ($p = 0.04$) and infiltrative invasion ($p = 0.03$).

Conclusions: HPV status and distribution had shown no correlation with worse prognosis of histological aspects, or predictive value for lymphatic metastasis in penile carcinoma.

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INTRODUCTION

Epidermoid carcinoma of penis (ECP) is a rare neoplasm, but with high incidence in underdeveloped countries (1). In Brazil, the incidence varies from 2.9 to 6.8 cases/100.000 inhabitants/year (2). In an epidemiological study in Pará State, the observed incidence was 5.7 cases/100.000 inhabitants/year (3).

Its etiology is associated with several risk factors, including presence of prepuce, bad hygienic habits, chronic dermatitis and smoking. How-

ever, the most extensively studied factor is human papilloma virus infection (HPV) (4).

HPV has more than 100 subtypes that can be classified as with low (HPV_{low}) or high risk (HPV_{high}) oncogenic potential (5). Its role in cervical uterine cancer is well established, but its association with ECP is still being discussed.

In patients with ECP, the presence of HPV varies from 15% to 80% suggesting that only a subgroup of these tumors is associated with HPV infection (6).

Natural history of the disease is characterized by progressive invasion of surrounding penile

tissues, and posteriorly metastatic dissemination to inguinal lymph nodes (6). Lymph node involvement is the prognostic factor of worst impact on prognosis of disease-specific survival (6) and the analysis of this involvement is very important in order to determine the cure of the disease.

Nowadays, the available methods for staging regional lymph node involvement have low accuracy. For example, Gonzaga-Silva et al., using gamma probe in the sentinel lymph node, showed sensitivity of only 25% and 42.8% of false negative results (7).

The physical exam of inguinal region (inspection and palpation) is also inconclusive, since half the cases with lymph node involvement does not present tumor. Also, 20% of patients without lymph node detected during physical exam present local micrometastasis (8).

Inguinal lymph node involvement is the most accurate method of staging to diagnose occult metastasis. However, it has significant morbidity and does not benefit a relevant subgroup of patients without metastasis (8).

In literature, several histological factors of the primary lesion were identified with predictive value of inguinal metastasis. These include: differentiation grade, tumor staging, and presence of neurovascular and lymph vascular invasion, with independent prognostic importance (9).

Although HPV infection is associated with the most aggressive histological subtypes, its value as a predictive factor of lymph node metastasis and prognosis is still debated (10).

The present study was conducted in a group of patients from Northern region of Brazil (Pará State), in order to evaluate the prevalence, distribution and association of HPV infection with histological pattern of worse prognosis in ECP and to determine its possible predictive value of lymph node metastasis.

MATERIALS AND METHODS

Patients

This study, as well as its consent inform, was approved by the Local Ethical Committee. Eighty-two patients with ECP were treated at the Hospital Ophir Loyola, Belém - Pará, from 2001 to 2008. The

patients were submitted to penile amputation and the analysis of the lymph nodes followed a protocol proposed by the Urologic Department of the Hospital. All patients were prospectively evaluated, by follow-up appointments, medical files charts or telephonic contact of relatives.

Treatment protocol

Lymph node resection was performed after 4 to 6 weeks of the penile surgery. During this interval patients received antibioticotherapy with fluoroquinolones. For the patients with palpable inguinal lymph nodes after the antibiotic treatment it was performed bilateral superficial lymph node resection with freezing histological samples. Patients with T2-T4 staging and/or G2-G3 differentiation grade (according to TNM 2009 UICC System of Staging and Pathology) were also submitted to superficial bilateral lymph node resection.

In the presence of tumor involvement of superficial lymph nodes, radical lymphadenectomy was performed. Pelvic lymphadenectomy was performed in all cases with deep inguinal lymph node invasion.

The surgery was not performed in patients with Tis or T1, G1 staging, without lymph vascular invasion or palpable lymph nodes. These patients followed a careful ambulatory schedule of surveillance for at least five years.

Pathological Analysis

All samples were revised blindly by a single pathologist. The tumors were retrospectively staged according to 2009 TNM System.

Lymph vascular invasion (LVI) was defined as the presence of tumor cells in the interior of lymphatic veins, blood veins or arteries. Perineural invasion (PNI) was determined as the presence of tumor tissue next to peripheral nerve branches. As for invasion, the tumor was considered "infiltrative", when it was observed the presence of branches of malignant cells within the stroma, or "pushing", when it was observed an interface between the tumor and the host cells.

Histological subtypes were classified as usual, condylomatous, verrucous, basaloid, papillary, sarcomatoid and acantholysis.

HPV Analysis

This part of the study was developed by the Papilloma Virus Laboratory of Instituto Evandro Chagas, a HPV referential laboratory of Brazilian Ministry of Health.

Parafin blocks were serially cut by microtome and the cuts were used for viral DNA detection.

Samples were submitted to polymerase chain reaction (PCR) using initially human beta globin G73 - 5'GAA GAG GGA AGG ACA GGT AC 3' e G74 - 5' CAA CTT CAT CCA CGT TCA CC 3' (PCR).

Viral gene specific reaction used the initiators GP5+ - 5' TTT GTT ACT GTG GTA GAT ACT AC 3' e GP6+ - 5' GAA AAA TAA ACT GTA AAT CAT ATT C 3', that were able to amplify a 150pb fragment of the L1 gene, that corresponds to a well preserved region of the virus genome.

After PCR, the identification of the virus was carried out using direct sequencing of the PCR product, using a capillary sequencer ABI 3130 Genetic analyser, Hitachi®.

The presence of HPV was classified in three subgroups:

HPVhigh - infection by one or more high risk virus

HPVlow - infection by one or more low risk virus

HPVmult - presence of more than one subtype of virus in the sample, whether of high or low risk.

Follow-up and statistical analysis

Follow-up of patients was carried out through periodic ambulatory evaluations, quarterly in the first two years after surgery, and then semiannually.

The presence or absence of HPV infection, as well the presence of subgroups HPVhigh, HPVlow and HPVmult were related to several clinical and pathological variables, using the Chi-Square method or Fisher exact test.

The presence of inguinal metastasis was correlated to: pT, G, LVI, PNI, Infiltrative, Pushing, histological subtype and HPV status, using univariate and multivariate analysis by Cox proportional hazard method.

The analysis of inguinal disease-free interval (IDFI) in five years used the Kaplan-Meier method

and the log Rank test. SPSS® 13.0 was used for all analyses, and the result was considered significant if $p < 0.05$.

RESULTS

Median age of patients was 58 years (22-91 years) and follow-up ranged from 1 to 71 months (median 20 months).

Presence of HPV DNA

HPV DNA was detected in 60.9% (50/82) fixed samples in paraffin. Viral distribution was: HPV11 (64%), HPV6 (32%), HPV16 (30%), HPV53 (18%), HPV33 (4%) and subtypes 18, 68, 45, 51, 52 and 58 (2%).

Subgroups HPVhigh, HPVlow and HPVmult were identified in 12/50 (24%), 25/50 (50%) and 25/50 (50%) respectively. Viral types that predominated in each group were 16, 11 and 11.

Histological characteristics of subgroups HPV positive (HPVhigh, HPVlow and HPVmult) and HPV negative

Patients without HPV infection had predominantly infiltrative invasion. HPV positive group was correlated to more differentiated tumors (Table-1).

HPVhigh was associated more frequently to basaloid subtype, and rarely was present in patients with usual pattern. There was also a low prevalence of HPVhigh in patients with more advanced penile carcinoma (Table-1).

Most patients with usual pattern were infected by HPVlow. Condylomatous type was associated more frequently to HPVmult infection (Table-1).

Multiple logistic regression analysis did not show any histological variable independently related to status or HPV subgroup.

Inguinal metastasis-free survival in five years and risk factor, according to histological patterns

It was observed 56% of inguinal involvement of metastasis (46/82 patients). Inguinal metastasis-free survival (IMFS) probability in five years showed no difference between the positive and negative HPV groups (34% x 29%, $p = 0.45$) - Figure-1.

Table 1 - Histopathological characteristics of 82 patients with penile cancer according to HPV positive (n = 50) or negative (n = 32), HPVhigh (n = 12), HPVlow (n = 25) and HPVmult (n = 25) groups.

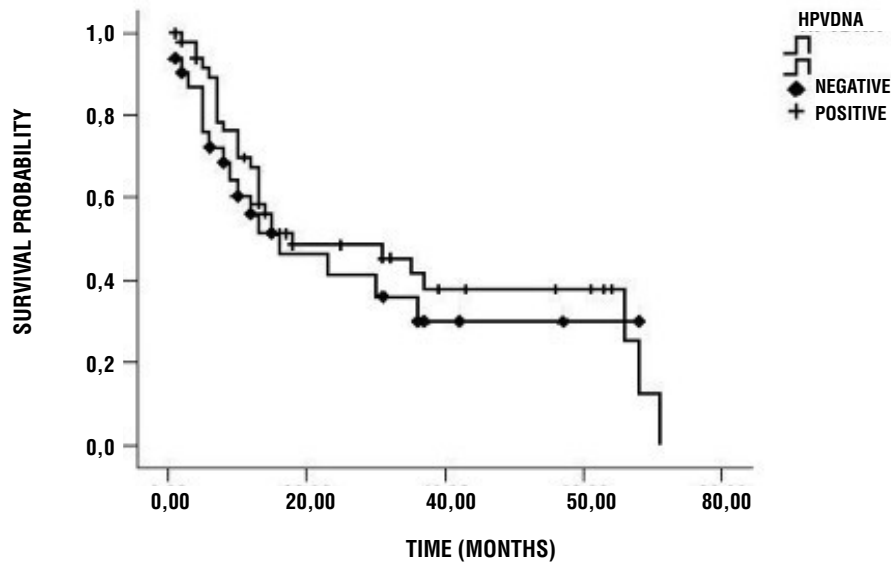
DNA-HPV									
	HPV+	HPV-	p	HPVHigh	p	HPVlow	p	HPVMult	p
TOTAL	50 (60.9)	32 (39)		12 (50)		25 (50)		25 (50)	
T1a	14 (28)	8 (72)		7 (58)		5 (22)		6 (22)	
T1b-T4	36 (72)	24 (75)	0.73 ¹	5 (42)	0.01 ²	2 (7)	0.35 ¹	1 (7)	0.70 ²
Grades 1 and 2	36 (72)	15 (46)		7 (58)		18 (72)		19 (76)	
Grade 3	14 (28)	17 (53)	0.02 ¹	5 (41)	0.75 ¹	7 (28)	0.22 ¹	7 (28)	0.08 ¹
LVI	8 (16)	3 (9)	0.39 ¹	3 (11)	0.20 ¹	3 (11)	0.80 ¹	3 (11)	0.80 ¹
PNI	11 (22)	7 (21)	0.98 ¹	1 (18)	0.21 ¹	6 (18)	0.76 ¹	5 (18)	0.77 ¹
INVASION									
Infiltrative	24 (48)	23 (71)	0.03 ²	4 (47)	0.06 ¹	12 (47)	0.25 ¹	13 (47)	0.51 ¹
Pushing	26 (52)	9 (28)		8 (35)		13 (35)		12 (35)	
SUBTYPE									
Usual	37 (74)	23 (71)	0.83 ¹	5 (60)	0.000 ²	23 (60)	0.01 ¹	18 (60)	0.87 ¹
Condilomatous	3 (6)	1 (3)	0.55 ¹	1 (4)	0.54 ²	0 (4)	0.17 ¹	3 (4)	0.04 ²
Verrucous	1 (20)	2 (6)	0.31 ¹	1 (3)	0.35 ¹	0 (3)	0.24 ¹	0 (3)	0.24 ¹
Basaloid	4 (8)	0 (0)	0.10 ¹	3 (4)	0.009 ²	1 (4)	0.80 ¹	1 (4)	0.80 ¹
Papillary	2 (4)	3 (9)	0.32 ¹	2 (5)	0.15 ¹	0 (5)	0.12 ²	1 (5)	0.59 ¹
Sarcomatoid	2 (4)	3 (9)	0.32 ¹	0 (5)	0.33 ¹	0 (5)	0.12 ¹	2 (5)	0.63 ¹
Acantholysis	1 (2)	0 (0)	0.42 ¹	0 (1)	0.67 ¹	1 (1)	0.12 ¹	0 (1)	0.50 ¹
pN	29 (58)	17 (53)	0.66 ¹	6 (46)	0.64 ¹	15 (46)	0.63 ¹	12 (46)	0.32 ¹

The values in parenthesis indicate percentage

1: Chi square test;

2: Fisher exact test.

Figure 1 - Inguinal metastasis-free survival after treatment of the primary tumor showed no significant differences between hpv positive tumors (n50/82) or negative hpv tumors (n32/82), $p = 0.45$.



Likewise, there was no statistical significant difference of IMFS among the groups HPVhigh, HPVlow and HOVMult (Table-2).

Univariate analysis of IMFS in five years, according to histopatological patterns, showed that stage $T \geq T1b-T4$ (HR = 5.76), LVI (HR = 2.90), PNI (HR = 2.88), and infiltrative invasion (HR = 2.68) were, decreasingly, strong predictors of inguinal metastasis, respectively 82.6% (38/52), 100% (11/11), 94.4% (17/18) and 70.2% (33/47) (Table-2).

Only sarcomatoid subtype was related to regional metastasis (4/5). Papillary subtype showed negative correlation to metastasis in 100% (0/5).

Multivariate analysis showed that $T \geq T1b-T4$, LVI and infiltrative invasion were strong independent predictors of inguinal metastasis (Table-3).

DISCUSSION

The present study is the first scientific report of the presence of HPV and its genotype variants correlated to ECP in a northern state of Brazil, a region with high prevalence of the disease.

In this sample, HPV prevalence in patients with ECP was 60.9%, in accordance to literature. It is reported a variation of 14% to 100% (11). Miral-

les-Guri et al. metanalysis in 2009, comprising 30 studies and 1266 patients with ECP reported a HPV DNA prevalence of 47.9%. In South America, viral DNA was specifically found in 40.7% of tumors (12).

As for the genotype distribution, in our sample there was a clear prevalence of types 11 and 6, contrary to the previous report of Bezerra et al., that reported an absolute prevalence of type 16 (12). We also registered infection by multiple HPV genotypes in 30.4% (25/50), contrary to the study by Krustup et al., that did not show multiple viral infections in their sample (13).

Variations of the reported prevalence of DNA HPV are mainly due to different techniques of sampling, studied population, detection methods, tumoral tissue storage and inclusion of multiple histological subtypes (14).

The role of HPV in epithelium carcinogenesis of uterine cervix is well established. In more than 95% of patients it is observed several high risk subtypes of virus infection (15); however, in ECP, the viral infection is not mandatory and there are several other causes not related to HPV infection (15). Relation of genital tumors and HPVhigh is stimulating research and interest of the biological potential viral interference on metastasis and disease prognosis.

Table 2 - Inguinal metastasis-free survival index in 5 years and risk rate according to clinical and pathological variables – univariate analysis

	Number of patients	IMFS in 5 years (95%IC) (%)	p Value (Log-Rank test)	Risk rate (IC95%)
pT				
T1a	23	59 (45 - 73)	0.002	5.76 (1.78 - 18.64)
T1b-T4	57	26 (19 - 33)		
GRADES				
1-2	51	34 (25 - 43)	0.42	1.27 (0.70 - 2.31)
3	31	28 (18 - 39)		
LVI				
Present	11	14 (3 - 25)	0.0004	2.90 (1.44 - 5.81)
Absent	71	36 (28 - 43)		
PNI				
Present	18	14 (6 - 22)	0.000	2.88 (1.54 - 5.37)
Absent	64	39 (30 - 47)		
INVASION				
Infiltrative	47	23 (16 - 31)	0.003	2.68 (1.38 - 5.22)
Pushing	35	46 (35 - 57)		
SUBTYPE				
Usual	60	33 (25 - 41)	0.55	0.81 (0.39 - 1.65)
Condilomatous	4	38 (10 - 66)	0.52	0.53 (0.73 - 3.88)
Verrucous	3	9 (2 - 15)	0.99	1.00 (0.13 - 7.38)
Basaloid	4	12 (9 - 15)	0.24	1.96 (0.60 - 6.45)
Papillary	5	9 (4 - 13)	0.02	0.04 (0.00 - 15.59)
Sarcomatoid	5	9 (4 - 13)	0.02	3.02 (1.05 - 8.64)
Acantholysis	1	7 (7 - 7)	0.14	3.88 (0.51 - 29.2)
HPV				
Positive	50	34 (25 - 43)	0.45	0.79 (0.43 - 1.46)
Negative	32	29 (18 - 40)		

Table 3 - Inguinal metastasis-free survival index in 5 years and risk rate according to clinical and pathological variables - multivariate analysis.

COX multivariate regression analysis – independent risk factors for inguinal metastasis		
Variables	Risk rate (IC95%)	“p” value
pT1axpT1b-4 (pT1b-4)	2.67 (1.16 - 6.15)	0.02
LVI +	2.09 (1.03 - 4.22)	0.03
Infiltrative invasion	2.00 (1.00 - 4.03)	0.04

Several authors tried to relate viral infection and histological pattern of worse prognosis (16-18). Scheiner et al. (16), Lont et al. (17) and Kirrander et al. (18) did not find such evidence. However, Gregoire et al. (19) and Krustup et al. (13) reported a significant statistical association between HPV_{high} infection and high risk tumors. Our studied showed that the only positive association was between the prevalence of HPV_{high} in 75% of basaloid tumors, with worse prognosis. Cubilla et al. (20) reported the same association.

Guimaraes et al. evaluated 14 cases of basaloid tumors, and reported 50% of inguinal metastasis. In our sample we observed 75% (3/4 patients) (21). Despite the small number of patients with this histological subtype of tumor, we believe that this association should be more investigated hereafter.

The recommendations for lymphadenectomies without the presence of inguinal lymph node disease are based upon predictive factors related to the primary lesion, mainly tumor stage and cellular differentiation grade. However, overtreatment can reach 82% of cases (22). In our sample, this was observed in 88% (30/34) even with the use of a strict protocol. It is necessary to search for non-invasive biomarkers with good accuracy, to predict the risk of metastatic lymph node involvement in patients with penile cancer (22,23). In our series, there was no association between the presence or distribution of HPV genotype with lymph node disease, as reported by other authors (13,19). At present, there are no evidences indicating the use of HPV detection in order to decide to perform lymphadenectomies.

Surprisingly, Lont et al., although failed to associate HPV status and lymph node metastasis, showed better specific-disease survival in patients with ECP associated with high risk virus ($p = 0.03$) (17). In order to avoid bias in our study, we only evaluated inguinal disease-free survival (IDFI), that did not show differences between HPV positive and negative groups ($p = 0.45$).

Several pathological and molecular risk factor are being described in order to improve lymph node evaluation (24,25). Among pathological predictive factors, only PNI, LVI and histological grade are independent factors to predict lymph node involvement (25). In our study, tumor stage \geq T1b-T4, LVI, PNI, infiltrative invasion and sarcomatoid histological subtype are the histological factors related to lymph node metastasis in a univariate analysis.

There was no significant difference among the cellular differentiation grades as prognostic factors, in spite of the reduced sample. However, pathological heterogeneity, with more than a grade presented in the same tumor is rarely considered in different studies.

Multivariate regression analysis showed that only \geq T1b-T4 tumors, LVI and infiltrative pattern were independent factors, and T stage was the most important (HR = 2.67, $p = 0.02$). The reported differences in several series are mainly caused by methodology, therapeutic approach and heterogeneity of the studied population. These limitations must be contoured in order to obtain a better validation of these risk factors for inguinal metastasis.

CONCLUSIONS

Observed HPV prevalence was similar to previous studies. However, there was a predominance of the low risk viral group. There was no significant association between HPV status or HPV subgroups and histological worse prognostic factors. HPV detection had no use as predictive value of inguinal metastasis. Tumor staging, lymph-vascular invasion and infiltrative pattern were independent risk factors for lymph node involvement.

CONFLICT OF INTEREST

None declared.

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