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Original Research Article

Human papilloma virus associated carcinoma penis: a comparative study for histopathological correlation and outcome analysis

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ABSTRACT

Background: Penile cancer prevalence is higher in the developing countries like Africa, Asia and South America as compared to western countries. HPV (human papilloma virus) DNA is detectable in about 50% of all penile cancer in India. The aim of the study was to compare tumour depth, lymph node metastasis and lymphovascular invasion and other high risk features in HPV positive penile cancer patients to HPV negative penile cancer patients.
Methods: This prospective, comparative study was done at Mahavir cancer sansthan a tertiary cancer centre in Patna (India). Biopsy proven cases of HPV positive and HPV negative penile cancer were compared for histological parameters and disease free survival. Statistical analysis was performed using NCSS 12 version 12.0.5 software. Kaplan- Meir survival analysis was done for disease free survival.
Results: On bivariate analysis, factors associated with HPV positivity were histological subtype (p=0.00001), grade of tumour (p=0.00698), depth of invasion (p=0.00001) and P16 status (p=0.00001). Depth of invasion (p=0.0499) and P16 status (p=0.00001) were the only independent factors associated with HPV status on multivariate analysis. There was no significant difference in 2 year disease free survival between the two groups, 91.57% vs 87.95% (p=0.4166).
Conclusions: Large proportion of penile cancer is associated with HPV in India. HPV associated penile cancer are highly invasive with predominant warty–basaloid histology. P16 immunostaining is strongly associated with HPV tumour. There is no survival advantage in HPV associated penile cancer as compared to HPV negative penile cancer.

Keywords: Penile cancer, India, HPV positive, Disease free survival, P16 immunostaining

INTRODUCTION

Penile cancer represents 20-30% of all cancers diagnosed in men who live in Asia, Africa, and South America.[1,2] In urban India, the age-adjusted incidence of penile cancer ranges from 0.7-2.3 cases per 100,000 men. In rural India the rate of penile cancer is 3 cases per 100,000 men, accounting for more than 6% of all malignancies in this population.[3] According to ICO (catalan institute of oncology) information register for HPV, penile cancer associated with HPV has crude incidence rate of 0.3-1.8%.

Approximately 60-100% of penile intraepithelial lesions are HPV DNA positive. HPV DNA is detectable in about 50% of all penile cancer in India.[4] The two most important risk factors for penile cancer are HPV infection and phimosis, which increases with the number of sexual partners, a history of genital warts, and concomitant sexually transmitted disease. HIV infection, poor hygiene, smegma, balanitis, phimosis, paraphimosis, lichen scleroses, immunosuppression and PUVA treatment are associated factors.

The estimated overall prevalence of HPV in penile cancer is 42% to 48% with the most commonly involved HPV subtypes being HPV 16 and 18.[5] HPV DNA has been found in approximately 90% of cases of dysplasia and 100% condylomata of penis.[6] More than 95% of penile squamous cells originate from the glans, preputium or

sulcus coronaries. Among non-HPV variant SCC, majority are not-otherwise-specified (NOS) (45-65%), verrucous carcinoma (2-3%) and papillary carcinoma (5-10%), which occurs in older people.[7]

Among HPV associated penile SCC subtypes, basaloid carcinoma is more common, predominantly in younger men. Lymph node metastasis is the main predictive factor for unfavourable prognosis in patients with penile cancer. The histological subtypes, histological grade and lymphovascular invasion are the other important variables of the primary tumour predicting inguinal lymph node metastasis. Prognostic significance of HPV in penile cancer has been proven in few recent studies; however data from Indian subcontinent are lacking. More data are needed to consolidate this.[8]

In the present study, high risk HPV associated penile cancer patients were analysed for histomorphological subtypes, lymph node metastasis, high risk features and other prognostic factors. This was compared to HPV negative penile cancer group.

METHODS

This prospective study was done at Mahavir cancer sansthan Patna (India) between February 2016 to June 2018. Patients with suspected penile cancer were examined in the department of surgical oncology. Patients with penile squamous carcinoma stage 1 to stage 3 with ECOG performance level 0 and 1 were selected for the study. Informed and written consent were taken individually prior to study. Study was approved by ethical committee of the institution.

Biopsy proven invasive squamous cell carcinoma who were fulfilling inclusion criteria were selected for the study. A total of 226 patients of suspected penile cancer patients visited surgery outpatient department during February 2016 to June 2017. Patients after incision biopsy, were consecutively tested for presence of high risk HPV DNA (type 16, 18) by real time PCR (polymerase chain reaction) method. Our required minimum number of patients in this double arm study was 83 in each group. 29 patients were excluded from the study, who had not fulfilled inclusion criteria. First 83 patients in each group were selected for the study.

For PCR analysis, formalin fixed paraffin-embedded biopsy specimen of all patients was tested for presence of high risk HPV DNA type 16, 18. If any type was positive, patient was considered positive for HPV. Clinically non-palpable inguinal nodes were evaluated by ultrasonography (USG) of bilateral groin. USG abdomen was done to detect any pelvic node. All patients were then categorized as per AJCC TNM staging system 8th edition and were treated with partial or total penectomy and bilateral groin and pelvic node dissection as and when required. For low risk group (T1, grade 1 or 2 and absent lymphovascular invasion, N0), only partial penectomy was done and patients were put on surveillance, while high risk group (T2, T3, grade 3 and positive lymphovascular invasion, N+) were treated by partial/total penectomy and bilateral groin node dissection. Intraoperative suspected groin nodes were sent for frozen section and if found positive for metastasis, then complete ilio-inguinal lymph node dissection was done. After surgery specimen was sent for histopathological examination. Histopathological results were categorized into following points -

- *Squamous cell carcinoma subtype:*
  - Not otherwise specified (NOS),
  - Verrucous carcinoma,
  - Warty carcinoma,
  - Basaloid carcinoma.
- Lymphovascular invasion.
- Perineural invasion.
- Grade- I-well differentiated,
  - II-Moderately differentiated.
  - III-Poorly differentiated.
- Depth of invasion- 8 mm was put cut off value.[9] More than 8 mm depth was considered highly invasive disease.
- Lymph node positivity.
- Pathological T stage.
- *P16 sensitivity:* P16 IHC staining was done on paraffin wax prepared specimen. Positive staining was noted for each group of patients. More than 10% stained cells were considered positive staining. Sensitivity of P16 immunostain for HPV positive tumour was determined.

After discharge from hospital, patients were followed every 3 monthly. Minimum follow up period was 12 months. Patients were noted for any local or metastatic recurrence. Any suspicious recurrence was proven by FNAC or biopsy. Number of patients was noted for lost to follow up and death either due to disease or any unrelated cause. Kaplan-Meier survival analysis was done and disease free survival between two groups was compared by log rank test.

Statistical method

HPV positive group was compared to negative group for different histological parameters as histological subtype, LVI, PNI, Lymph node status, P16 sensitivity, depth of invasion and grade of disease.

The chi-square test was used to assess the association between different variables. Bivariate and multivariate analysis was done for each parameter. Statistical analyses were performed using NCSS 12 version 12.0.5 statistical software. Statistical significance was considered at $p<0.05$. Data was entered into excel spread sheet. Microsoft word and excel was used to generate graph and tables.

RESULTS

Demography

In this study, a total of 226 patients of carcinoma penis visited surgery OPD from February 2016 to June 2017. Total no. of HPV positive cases were 103, so incidence of hpv in ca penis in this hospital was 45.5%. Incidence of HPV type 16 was 90.3% and incidence of HPV type 18 was 41.7% (Table 1).

Mean age±SD of presentation was 55.09 ±14.41 years and median age was 55 years. In HPV positive group there was 60.2% patients of >50 yrs age group as compared to 62.6% patients of >50 yrs age group in HPV negative group. In both groups, majority of patients were of lower socioeconomic status (60% v/s 63.8%) respectively in HPV positive and negative group. In HPV positive group number of patients in stage I, II, III patients were 32.5%, 24.1% and 43.3% while in HPV negative group it was 38.5%, 24.1% and 37.3% respectively (Table 2).

Table 1: Proportion of HPV in Ca penis patients in this hospital.

No. of HPV negative patients	No. of HPV positive patients			Total no. of Ca penis patients
	HPV 16	HPV 18	Both type	
123	60	10	33	226

Table 2: Bivariate comparison between HPV positive and HPV negative group.

Variables	Group	HPV status		Chi square value	P value
		Positive	Negative		
Age (in years)	<50	33	31	0.1017	0.7497
	≥50	50	52		
Socioeconomic status	Lower	49	53	0.9	0.6376
	Middle	33	28		
	Upper	1	2		
Clinical stage	Stage I	27	32	0.7969	0.6713
	Stage II	20	20		
	Stage III	36	31		
Subtype	NOS	49	50	28.5692	0.00001
	Verrucous	5	26		
	Warty	11	1		
	Basaloid	18	6		
pT stage	T1	26	31	2.1315	0.34447
	T2	34	25		
	T3	23	27		
[a] LN status	Present	25	24	0.029	0.864883
	Absent	58	59		
Grade	I	24	41	10.0118	0.006698
	II	50	40		
	III	9	2		
[b]DOI (in mm)	≤8 mm	27	65	35.2092	0.00001
	>8 mm	56	18		
LVI	Present	19	25	1.1133	0.2913
	Absent	64	58		
PNI	Present	8	11	0.5349	0.46455
	Absent	75	72		
P16 status	Positive	75	16	84.6661	0.00001
	Negative	8	67		

a- lymph node b- depth of invasion.

Histopathological characteristics

Histological subtype, pathological T stage, pathological lymph node positivity, grade, depth of invasion, lymphovascular invasion, perineural invasion and P16 stain positivity was used to compare their association with HPV infection.

On bivariate analysis, factors associated with HPV positivity were histological SCC subtype (p=0.00001),

grade of tumour (p=0.00698), depth of invasion (p=0.00001) and p16 status (p=0.00001). Other factors like clinical stage (p=0.6713), pathological T stage (p=0.3444), lymph node status (p=0.8282), LVI (p=0.2913), PNI (p=0.46455) were not significantly associated with HPV status (Table 2).

By using multivariate logistic regression analysis, only depth of invasion (p=0.0499) and p16 status (p=0.00001) were independently associated with HPV positive state (Table 3).

Table 3: Multivariate analysis of histopathological parameters.

Independent variable x	Regression coefficient b (i)	Standard errors b(i)	Wald Z value	Wald P value	Odds ratio exp (b(i))	Lower 95% confidence limit	Upper 95% confidence limit
Histological subtype	-0.09020	0.21305	-0.423	0.67202	0.91375	0.5077	0.32737
Grade	0.15107	0.45240	0.334	0.73843	1.16308	0.7352	1.03777
Depth of invasion	-1.04906	0.53522	-1.960	0.04999	0.35027	2.097	0.00005
P16 status	3.29952	0.48916	6.745	0.000001	27.09967	2.3408	4.25827

Kaplan-Meier survival analysis

Total numbers of cases followed were 83 cases in each HPV positive and HPV negative group, with minimum follow-up of 1 year. There was local/regional recurrence of disease in 7 cases among HPV positive group and 10 cases in HPV negative group. 9 cases were lost to follow up (5 in positive group and 4 in negative group). Disease free survival was compared between both groups after follow up within study period.

Median follow-up was 15 months (range 12-24). Mean survival in HPV positive group was 22.564 month as compared to 21.855 months in HPV negative group. Cumulative probability for disease free survival by high risk HPV status is presented in figure below (Figure 1). There was no significant difference in disease free survival between the high risk HPV positive and negative group: 91.57% v/s 87.95%.

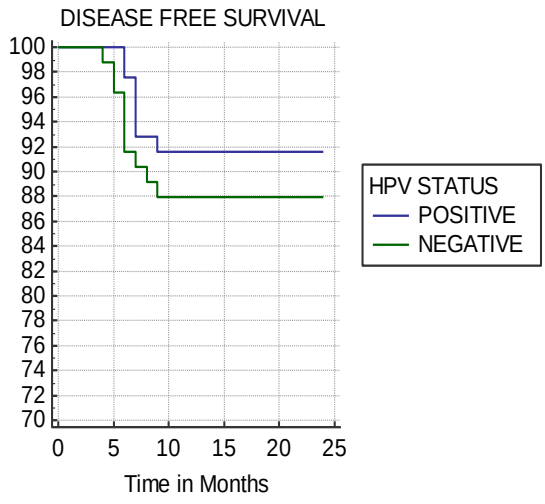

Figure 1: Kaplan – Meir survival analysis between HPV positive and negative group.

Comparison of survival curves (log rank test)

Chi square value =0.6599, DF =1.
Log rank test significance, p=0.4166

DISCUSSION

Proportion of HPV in Ca penis

Proportion rate of HPV associated Ca penis was found to be 45.5% in this hospital. According to ICO information register for HPV (2015), HPV DNA is detectable in 50% of Ca penis patients in India.[4] There is wide variation in incidence rate across the globe due to geographical condition, socioeconomic status and sexual practices. In developing countries like India, HPV infection is prevalent in low socioeconomic status with poor hygiene and increased number of sexually transmitted disease due to lack of sex education. In both groups majority of cases were of lower socioeconomic status. Increased incidence of Ca penis in low socioeconomic status is well established as reported high prevalence in Asia, Africa and Brazil.

Clinical stage

In developing world, patients with Ca penis present at more advanced stage either because of lack of awareness, embarrassment, fear or lack of access to specialized care. In this study, there was no statistical correlation of HPV with clinical stage of the disease (p=0.6713).

***Histopathological parameters related to HPV* (on bivariate and multivariate analysis)**

Depth of invasion has direct correlation with lymph node metastasis in Ca penis. It is used as a predictor of lymphatic metastasis which characterizes disease aggressiveness and impact on future survival. Whether HPV association has direct impact on depth of invasion in Ca penis, is largely unknown.

In our study, we found significant number of HPV positive Ca penis patients with highly invasive disease. This is consistent with presence of predominant aggressive histology (warty-basaloid) among HPV positive patients in our study.

Positive immunostaining for P16INK4a, a tumour suppressor protein, is regarded as a surrogate marker for HPV associated tumour. The strong relationship between HPV infection and increased P16INK4a expression has resulted in the use of P16INK4a immunostaining as a means of HPV testing.

In our study we found a good sensitivity (90.4%) of P16 for HPV associated tumour. Although correlation of P16 with other tumour adverse factors was not studied.

Disease free survival

In this study we could not find any significant survival benefit in HPV positive patients over HPV negative patients. Among different survival studies related to HPV associated Ca penis, only two studies described significant survival benefit in HPV positive patients.[10,11]

In contrast to the established role of HPV as a risk factor, little is known about its prognostic significance in penile SCC. In penile carcinoma, the prognostic significance of lymph node invasion on survival has been well established. Predictive histopathological features for lymph node metastasis include tumour grade, depth of invasion and LVI. In our study HPV was only associated with depth of invasion. In previous literatures, it has been mentioned that HPV positive tumour are high grade, deeply invasive with predominant warty-basaloid histology. In contrast few authors have described that increased survival of patients with HPV positive carcinoma may be owing to a lower degree of gross genetic alteration, as has been previously found in head and neck SCC.[11,12] The presence of virus in penile carcinoma may confer an increased immune surveillance, thereby making the HPV associated malignancy less aggressive.

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REFERENCES

1. Bleeker MC, Heideman DA, Snijders PJ, Horenblas S, Dillner J, Meijer CJ. Penile cancer: epidemiology, pathogenesis and prevention. World J Urol. 2009;27(2):141.
2. Hakenberg OW, Compérat EM, Minhas S, Necchi A, Protzel C, Watkin N. EAU guidelines on penile cancer: 2014 update. Eur Urol. 2015;67(1):142-50.
3. Available at: http://www.indiacancersurgerysite.com/penile-cancer-treatment-india.html. Accessed on 3 June 2019.
4. Human Papillomavirus and Related Diseases Report. Available at: www.hpvcentre.net/statistics/reports/XWX.pdf. Accessed on 18 June 2019.
5. Bansal A, Singh MP, Rai B. Human papillomavirus-associated cancers: A growing global problem. International Journal of Applied and Basic Medical Research. 2016;6(2):84.
6. Gross G, Pfister H. Role of human papillomavirus in penile cancer, penile intraepithelial squamous cell neoplasias and in genital warts. Med Microbiol Immunol. 2004;193(1):35-44.
7. Junker K, Hölters S, Hartmann A. Human Papilloma Virus, Histopathological, and Molecular Subtyping in Penile Cancer: Relevance for Prognosis and Therapy. European Urology Supplements. 2017;16(12):295-300.
8. Zhu Y, Zhang HL, Yao XD, Zhang SL, Dai B, Shen YJ, et al. Development and evaluation of a nomogram to predict inguinal lymph node metastasis in patients with penile cancer and clinically negative lymph nodes. J Urol. 2010;184(2):539-45.
9. Bhagat SK, Walter N, Gopalakrishnan G. Predicting inguinal metastases in cancer penis. Indian J Urol. 2006;22(4):351.
10. Prowse DM, Ktori EN, Chandrasekaran D, Prapa A, Baithun S. Human papillomavirus-associated increase in p16INK4A expression in penile lichen sclerosus and squamous cell carcinoma. Br J Dermatol. 2008;158(2):261-5.
11. Djajadiningrat RS, Jordanova ES, Kroon BK, van Werkhoven E, de Jong J, Pronk DT, Snijders PJ, Horenblas S, Heideman DA. Human papillomavirus prevalence in invasive penile cancer and association with clinical outcome. J Urol. 2015;193(2):526-31.
12. Lont AP, Kroon BK, Horenblas S, Gallee MP, Berkhof J, Meijer CJ, et al. Presence of high-risk human papillomavirus DNA in penile carcinoma predicts favorable outcome in survival. Int J Cancer. 2006;119(5):1078-81.

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