Prognostic value of HPV DNA in Urothelial Carcinoma of the Bladder: A Preliminary Report of 2-Year Follow-up Results

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Purpose: The association between the human papillomavirus (HPV) and anogenital carcinomas is well established. However, despite its anatomic adjacency, the relationship between HPV and urothelial carcinoma of the bladder (UCB) is less clear. Recent meta-analysis and case-control studies demonstrated a significant relationship between the presence of HPV DNA and UCB. The aim of this clinical study was to compare the 2-year follow-up results of HPV-positive and HPV-negative UCB patients to evaluate the prognostic value of HPV DNA positivity in UCB.

Methods: The study included patients with stage pTa and pT1 UCB who underwent polymerase chain reaction (PCR) analysis of HPV DNA between January 1 and November 30, 2018. Based on their PCR results, 19 HPV-positive and 38 HPV-negative UCB patients who had regular follow-up in our clinic were evaluated in terms of tumor recurrence and disease progression over a 2-year follow-up period.

Results: There was no significant difference between the groups in terms of age, follow-up time, smoking, or tumor grade (P = .576, P = .368, P = .080, and P = .454). Tumor recurrence was observed at least once in 47.3% (n=9) of the 19 HPV-positive patients and 36.8% (n=14) of the 38 HPV-negative patients (P = .445). There was no difference in disease progression between the groups during follow-up.

Conclusion: In our sample of UCB patients, the presence of HPV DNA was associated with a trend toward higher recurrence rate during the 2-year follow-up, though the difference was not statistically significant. No difference in disease progression was observed based on HPV DNA positivity.

Keywords: urothelial carcinoma; bladder; HPV; prognosis; PCR

INTRODUCTION

Human papillomavirus (HPV) is a double-stranded DNA virus and currently the most common sexually transmitted pathogen worldwide. According to epidemiological studies, the annual global prevalence of HPV is as high as 11.7%.[1] The main reason for this high prevalence is that most HPV infections are asymptomatic or subclinically controlled by host adaptive immunity and become undetectable over time. The oncogenic nature of HPV is another reason that it presents a serious global socioeconomic burden. HPV is one of the most important viruses implicated in infection-related cancers and is thought to be responsible for 7 to 8% of all human malignancies.[2] Over 200 different HPVs have been identified to date, of which more than 40 are responsible for anogenital infections and HPV-associated malignancies.[3,4] Squamous cell carcinoma is the most common histologic type of cancer associated with HPV due to HPV tropism for squamous epithelium. The relationship between HPV and cervical cancers, as well as anogenital and certain head and neck carcinomas, has been unequivocally demonstrated. HPV coexistence is reported in 96% of cervical cancers, 64% of anal cancers, 36% of penile cancers, and 41% of head and neck cancers.[5,6] However, the relationship between HPV and bladder cancer has remained a subject of controversy, despite its anatomic adjacency. Coexistence of HPV and primary bladder cancer has been reported at rates ranging from 0 to 100% (overall prevalence 16.8%).[7,8] This lingering uncertainty can be largely attributed to methodological limitations of previous studies, namely limited case series, lack of fresh tissue sampling, and not following a case-control design.[9,10] Therefore, Sarier et al.[11] recently conducted a case-control study with fresh samples and demonstrated a strong correlation between UCB and HPV infection (odds ratio 4.24,

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Received August 2020 & Accepted January 2021

Table 1. Demographic structure and distribution of follow-up results in patients with urothelial bladder carcinoma according to human papillomavirus (HPV) status.

	HPV- (n=38)	HPV+ (n=19)	*P*-value
Age (years)	64.7 ± 11.5	61.4 ± 13.9	.576
Male	31 (81.5%)	17 (89.4%)	.703
Female	7 (18.5%)	2 (10.6%)	
Smoking history	21 (55.2%)	15 (78.9%)	.080
High-grade tumor	18 (47.4%)	11 (57.9%)	.454
Low-grade tumor	20 (52.6%)	8 (42.1%)	
Clinical stage pTa	19 (50.0%)	13 (68.4%)	.180
Clinical stage pT1	19 (50.0%)	6 (31.6%)	
Tumor recurrence	14 (36.8%)	9 (47.3%)	.445
Follow-up time (months)	26.1 ± 6.9	27.5 ± 7.3	.368

Results expressed as mean ± standard deviation or frequency (percentage)

95% CI 1.63-12.34). However, to our knowledge there are no studies in the literature investigating the relationship between the presence of HPV DNA and bladder cancer prognosis. The aim of this clinical study was to compare 2-year follow-up results of HPV-positive and HPV-negative UCB patients to determine the prognostic value of HPV DNA positivity in UCB.

PATIENTS AND METHODS

Case selection and ethical approval

The study included patients who were diagnosed as having a primary or recurrent bladder tumor by ultrasound and/or cystoscopic examination in the urology outpatient clinic and underwent transurethral resection of bladder tumor (TUR-BT) between January 1 and November 30, 2018. Before surgery, first morning urine and urethral swab samples were collected for HPV DNA testing by polymerase chain reaction (PCR) analysis. Patients with clinical stage pT2 disease or higher and those with carcinoma in situ or non-urothelial carcinoma of the bladder according to their TUR-BT pathology results were excluded from the study. Patients with stage pTa and pT1 UCB were grouped according to their PCR results. Information regarding the patients' demographic characteristics, smoking history, and tumor grade were collected. Intravesical immunotherapy was administered to patients with intermediate- and high-risk tumors for 1 year following TUR-BT.[12]
During follow-up, control cystoscopy was performed every 3 months for the first year and every 6 months thereafter. A total of 19 HPV-positive and 38 HPV-negative UCB patients who regularly attended follow-up in our clinic were evaluated in terms of tumor recurrence and progression.
Local ethics committee approval was obtained (number 005/2018) and all patients provided written informed consent. The study was carried out in keeping with the Declaration of Helsinki.

Molecular analysis

First morning urine samples (15 mL) were obtained and urethral samples collected using a cotton-tipped swab before surgery. All samples were stored at -80°C until analysis.
DNA was extracted from the samples using the PREP-NA PLUS and PREP-GS PLUS extraction kits (DNA Technology®, Moscow, Russia) as per the manufacturer's instructions. The samples were analyzed for HPV DNA using a DT Prime 5 Real-Time PCR device (also manufactured/programmed by DNA Technology®). The samples were analyzed for low-risk (types 6, 11, 44) and high-risk (types 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 82) HPV.

Statistical analyses

All statistical analyses were performed using Open Epi® Version 3.01 (Atlanta, GA, USA). Shapiro–Wilk test was performed to determine whether the data followed normal distribution. Continuous variables were expressed as means and standard deviation, and comparisons between groups were done using Mann–Whitney U test. Chi-square test was used to evaluate relationships between categorical variables. *P* values less than 0.05 were considered statistically significant.

RESULTS

There were no statistical differences between the 19 HPV-positive UCB patients and 38 HPV-negative UCB patients in terms of age ($P = .576$), follow-up duration ($P = .368$), smoking history ($P = .080$), and tumor grade ($P = .454$) (**Table 1**). During follow-up, at least 1 tumor recurrence was observed in 47.3% (n = 9) of the HPV-positive patients and 36.8% (n = 14) of the HPV-negative patients ($P = .445$). No progression in tumor grade or clinical stage was detected in the patients during the follow-up period. High-risk HPV types were detected in 94.7% (n = 18/19) and low-risk HPV types were detected in 5.3% (1/19) of the HPV-positive patients. PCR revealed DNA from multiple HPV types in 4 patients. Distribution of the detected HPV types is shown in **Table 2**.

DISCUSSION

Bladder carcinomas are the fourth most common type of cancer in men and the seventh most common type of cancer in women worldwide, and the prognosis is poor in some cases despite advances in treatment.[13] Although factors such as tumor size, histological grade, and clinical stage are routinely used to predict recurrence and prognosis, these factors are usually inadequate to determine tumor course.[14] Therefore, studies are ongoing to investigate recurrence and prognosis prediction in bladder carcinomas and understand the effectiveness of treatment methods. The utility of various prognostic biomarkers such as epidermal growth factor receptor, p53, retinoblastoma (Rb), and p16 tumor suppressor genes has been investigated for prognostic stratification of patients.[14] However, none of these biomarkers has been widely adopted as a prognostic factor in bladder carcinoma. In the literature, one large study demonstrated that 39.1% of patients had tumor recur-

Table 2. Human papillomavirus (HPV) types detected by polymerase chain reaction in patients with urothelial carcinoma of the bladder

HPV Type	Patients, n (%)
Type 16	3 (11.5%)
Type 18	3 (11.5%)
Type 26	1 (3.9%)
Type 39	3 (11.5%)
Type 45	1 (3.9%)
Type 51	2 (7.7%)
Type 53	3 (11.5%)
Type 56	1 (3.9%)
Type 66	2 (7.7%)
Type 68	2 (7.7%)
Type 82	2 (7.7%)
Type 6	3 (11.5%)
Total	100%

rence without progression while 33.0% showed disease progression over a 10-year follow-up period despite intravesical immunotherapy/chemotherapy and surgical treatments.[15] Comparing the results of that study with our own, the recurrence rate among HPV-negative patients in our study was 36.4%, similar to the literature, while we observed a higher rate of 47.3% in HPV-positive patients.

HPV is known to act as an oncogene via viral oncoproteins E6 and E7.[16] E6 protein inhibits the function of the tumor suppressor protein p53, while E7 contributes to oncogenesis by inactivating RB1 protein, which is encoded by another tumor suppressor gene, Rb. The resulting disruptions in cell cycle control and DNA repair compromise the genomic stability of cells and increase the likelihood of malignant transformation.[17] E7 overexpression also leads to epigenetic remodeling of the p16 gene locus, which results in high levels of non-mutated functional p16.[18] However, as opposed to the normal consequence of p16 overexpression, which is cell cycle arrest, proliferation continues in HPV-transformed cells due to the nonfunctional Rb pathway.[19] Today, p16 is widely used as a surrogate biomarker in HPV-related anogenital and head and neck carcinomas. There are numerous studies investigating the relationship between HPV and urinary tract cancers. Unlike penile cancer, no significant relationship has been observed between HPV and prostate, testicular, or kidney cancers in previous studies.[20] However, this is not the case for bladder cancer. Two hypotheses have been proposed to explain the association between HPV and bladder cancer. One is that the urethra is the first point of contact during sexual transmission of the virus. The urethra provides a reservoir for the virus as well as a direct connection and natural route of entry to the urinary bladder from the genital area. The other hypothesis is based on the epithelial tropism exhibited by HPV.[21] The prognostic value of HPV infection in the cancers with which it is associated has also been investigated for many years. Published meta-analyses have indicated that HPV positivity is a favorable prognostic factor in cervical, anal, and head and neck cancers.[22,23,6] In addition, HPV positivity was associated with better response to radiotherapy and chemotherapy in head and neck cancers, resulting in better prognosis.[24] It is not clear how HPV positivity improves prognosis in these carcinomas. However, compared to HPV-positive cancers, highly metastatic HPV-negative primary cancers were found to have more aggressive p53 mutations that cause more severe growth dysregulation and poorer prognosis.[14] The present study is the first to evaluate the effect of HPV DNA on prognosis in UCB. Although we observed no statistically significant difference between the HPV-positive and HPV-negative groups in terms of disease progression at the end of follow-up, HPV-positive UCB patients tended to have higher frequency of tumor recurrence, unlike in other HPV-associated carcinomas. While this finding suggests that HPV-positive patients might have a higher risk of recurrent disease, at least in the short term, it must still be determined whether this is related to HPV infection. Cell character might be a factor in this. Cancers commonly associated with HPV are characteristically squamous cell carcinomas. However, UCB has different histopathological features. We believe that this study should be regarded as a preliminary study on the prognostic utility of HPV coexistence in urothelial carcinoma. In the future, investigating the expression of tumor suppressor genes such as p53, Rb, and especially p16, which is known to play a role in bladder carcinogenesis along with HPV, may help elucidate the prognostic value of HPV positivity.

Tumor grade is the most important predictor of progression in bladder cancer. Previous studies have also yielded discrepant results regarding the relationship tumor grade and HPV. HPV DNA positivity was correlated with low-grade tumors in a study by Tenti et al.[25], while Cai et al.[26] and Javanmard et al.[8] reported a correlation with high-grade tumors. In contrast to these studies, Sarier et al.[11] observed no statistical correlation between tumor grade and HPV DNA positivity. These three conflicting results show that it is too early to draw any conclusions about the relationship between HPV infection and tumor grade.

The distribution of HPV types detected in patients with UCB is another noteworthy finding from this study. Types 16 and 18 are known to be the predominant high-risk types responsible for the largest proportion of HPV-associated anogenital carcinoma cases.[20,27,28] However, developments in multiplex PCR technology have enabled the investigation of more genotypes, thus revealing a greater variety of high-risk genotypes.[26,29,30] In this study, types 16 and 18 together constituted only 23% of the detected HPV types. We consider this an important finding demonstrating the diversity of high-risk HPV types in UCB.

This study has some important limitations to address. Firstly, the case series could have been larger, which may have provided better coordination between clinical findings and statistical results. Secondly, this study evaluated 2-year results, but a follow-up period of at least 5 years would increase the significance of the study. In addition, investigating HPV-associated tumor suppressor genes in tumor tissues by immunohistochemical methods will be a guide to better demonstrate the prognostic value of HPV positivity.

CONCLUSIONS

HPV-positive and HPV-negative patients with pTa and pT1 UCB showed no significant difference in disease progression over a 2-year follow-up period. HPV-positive patients tended to have higher tumor recurrence rate, though the difference did not reach statistical significance. Future studies with larger series and longer follow-up times will provide more guidance on this subject.

CONFLICTS OF INTEREST

All authors declare no conflict of interest.

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