Human Papilloma Virus DNA in Tumor Tissue and Urine in Different Stage of Bladder Cancer

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Purpose: There are some previous reports on the relationship between bladder cancer pathological grades and HPV detection. To determine the Human Papilloma Virus(HPV) DNA in tumor Tissue and Urine in Different Stage of Bladder Cancer conducted this study.

Materials and Methods: Polymerase chain reaction (PCR) was used to detect general HPV and HPV16 and 18 subtypes in 110 bladder tumor tissue and urine specimens of patients with TCC of bladder between January 2014 to May 2016 that underwent transurethral resection of bladder tumor. Exclusion criteria were genital wart and cases with immunosupression.

Results: Mean age of 110 patients was 61.6±10 years and fourteen (12.7%) of patients were female. PCR for general HPV primer in bladder tumor tissue was positive in 3 (9.4%), 22 (38.6%) and 15 (71.4%) of Ta, T1 and T2 bladder tumors, respectively ($P < 0.001$). PCR for HPV16 in bladder tumor tissue was positive in 2(6.3%), 10 (17.5%) and 13 (61.9%) and PCR for HPV18 in bladder tumor tissue was positive in 1 (3.1%), 14 (24.6%) and 12 (57.1%) of Ta, T1 and T2 bladder tumors, respectively ($P < 0.001$, $P < 0.001$). Thirty seven (33.6%) of urine specimens were positive for general HPV using PCR and HPV16 and 18 subtypes were positive in 17 (15.5%) and 14 (12.7%) of urine specimens, respectively.

Conclusion: HPV infection may be associated with higher stages and grades of bladder carcinomas. Urine sampling for HPV detection is as reliable as tumor tissue sample which could be considered for prognostic and follow up implications.

Keywords: human papilloma virus; bladder transitional cell carcinoma; cancer grade; cancer stage

INTRODUCTION

One of the most common sexually transmitted viruses worldwide are Human papilloma Viruses (HPVs)[1]. Many epidemiological studies described that the prevalence of HPV among healthy men, is as high as that among healthy women[2]. It has been proposed that HPV is the most important risk factor for development of carcinoma in urogenital system[3].

Recent researches have shown that prevalence of HPV infection in subjects with bladder Transitional Cell Carcinoma (TCC) varies between 2% to 81.3% [4,5].

A study from Iran by using polymerase chain reaction (PCR), has showed the presence of HPV in 35.6% of TCC tissue specimens[6]. There are some previous reports on the relationship between pathological grades of bladder carcinoma and HPV detection. Tenti et al. has described that HPV prevalence in 79 samples of bladder carcinoma was 32.9%, and HPV infection was frequently found in low-grade (grade 1) tumors compared with high-grade tumors[7]. Badawi et al. also mentioned that HPV was detected in 44.4% of bladder carcinoma cases, which tended to be frequent in low-grade tumors[8]. Our previous study showed that HPV was positive in 8 (38.1%) of grade 1, 10 (47.6%) in grade 2, and 3 (14.3%) in grade 3 carcinomas[6]. Conversely, another study investigated the prevalence of HPV among the bladder wash samples in the patients with bladder carcinoma, and found positive correlation between

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Table 1. The association between patient characteristics and grade and stage of bladder tumor

		Age (± SD)	Male (%)	Cigarette smoker (%)	BCG therapy (%)	Total (%)
Stage						
	pTa	59.9 ± 8.8	26 (81.3)	16 (50)	10 (31.3)	32 (100)
	pT1	61.3 ± 11	50 (87.7)	40 (70.2)	22 (38.6)	57 (100)
	pT2	65 ± 9.2	20 (95.2)	12 (57.1)	12 (57.1)	21 (100)
	P value	0.2*	0.35**	0.17**	0.18**	
Grade						
	Low grade	61.8 ± 11.1	50 (83.3)	38 (63.3)	18 (30)	60 (100)
	High grade	61.3 ± 8.9	46 (92)	30 (60)	26 (52)	50 (100)
	P value	0.79***	0.25**	0.72**	0.019**	

*Kruskal-Wallis test,** Fisher's exact test, ****t*-test

HPV-positive rate and the pathological grade[9].
The prognostic implication of HPV infection in bladder cancer survival was suggested by some studies[10,11]. The aim of this study was to assess the correlation between urine and bladder tumor tissue HPV infection in Different Stages[12] of Bladder Cancer.

MATERIAL AND METHODS

Patients and samples

A total number of 110 patients who underwent transurethral resection of bladder tumor (TURBT) between January 2014 to May 2016 with the diagnosis of bladder TCC entered our study. Exclusion criteria were genital wart (according to physical examination and medical history) and cases with immunosupression (history of chemotherapy and high dose corticosteroids medication in long time). After insertion of resectoscope sheath, 10 ml of urine was collected and transported to laboratory under sterile condition. Bladder tumor tissue specimens after TURBT were evaluated histopathologically for grading[12] ,and staging and analyzed by PCR.

Sample preparation and DNA extraction

Formalin-fixed, paraffin-embedded bladder tumor tissue specimens and urine samples (urine was frozen in molecular biology research center) were collected and transported to molecular biology research center of Shahid Beheshti University of Medical Sciences under a well-controlled condition. Tissues were finely chopped (5-10 µm) using sterile microtome blades and digested in lyses buffer (0.33 M sucrose, 10 mM Tris bas, 5 mM MgCl2, 2% Titon X-100) at 37°C for 4 hours. About one ml of each urine sample also centrifuged at 10000 g for 5 min and 100 UL of protease buffer was added to the sediment. Phenol-chloroform extraction and ethanol precipitation were performed. The precipitated DNA was suspended in distilled water and used for amplification.

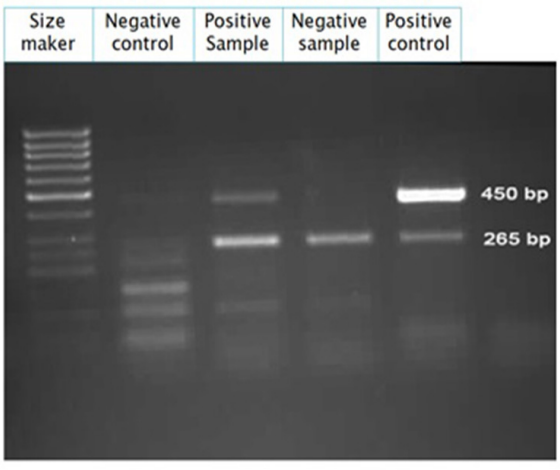

Figure 1. PCR experiment included samples with reference plasmids as positive controland several samples lacking template DNA as contamination controls.

Polymerase chain reaction

In this reaction, PCR amplifications were carried out in 50 µl volumes of a reaction mixture containing 1.5 mM MgCl2, 0.1 mM of each dNTP, 20 pico mol of each primer, 0.1 µg DNA, 1.25 units of Taq DAN polymerase and 1× PCR buffer. The sequences of primers were; HPV PCR Kit A101122 ,HPV-118 Detection kit A101192 ,HPV-16 Detection kit A101182. Initial 4 min denaturation step at 94 °C was followed by 30 cycles of amplification with an automated thermal cycler machine. Each cycle included a denaturation step at 94 °C for 30 s, an annealing step at 38 °C for 30 s, and an elongation step at 72 °C for 90 s, with a final elongation step was prolonged for further 4 min. Each PCR experiment included samples with reference plasmids as positive control (**Figure 1**) and several samples lacking template DNA as negative controls[10,11]. The reference plasmids were; HPV 450 bp, HPV-18 331 bp, HPV-16 246 bp and internal Control 260 bp.

Electrophoresis

PCR products were analyzed by electrophoresis on 2% agarose gels stained with ethidium bromide and visualized on an ultraviolet imager at 254 nm.

Statistical analysis

Statistical analysis was done with SPSS software (SPSS Inc., Chicago, IL, version 18.0) using Mann-Whitney, t-test, Kruskal-Wallis and fisher's exact tests as appropriate. The level of significance was established at $P < 0.05$.
Informed consent was obtained from all patients, also benefits and complications were described for them before entering the study. This study was designed and approved by ethical committee of Shahid Beheshti University of Medical Sciences.

RESULTS

Ninety six (87%) of patients were male, the mean (±SD) age was 61.6 ± 10 years (ranged between 32 to 85 years). Forty four (40%) patients had previous history of BCG therapy. Sixty eight (62%) patients were cigarette smoker. According to the pathologic pT stage,

Table 2. Association between stage and grade of bladder TCC and HPV, HPV 16 and 18 infections

		HPV 16 DNA	HPV 18 DNA	General HPV DNA	Total
Stage					
	pTa	2 (6.3%)	1 (3.1%)	3 (9.4%)	32 (100%)
	pT1	10 (17.5%)	14 (24.6%)	22 (38.6%)	57 (100%)
	pT2	13 (61.9%)	12 (57.1%)	15 (71.4%)	21 (100%)
	P value*	< 0.001	< 0.001	< 0.001	
Grade					
	Low grade	3 (5%)	3 (5%)	9 (15%)	60 (100%)
	High grade	22 (44%)	24 (48%)	31 (62%)	50 (100%)
	P value*	< 0.001	< 0.001	< 0.001	

* Fisher's exact test

cases were classified into: pTa in 32 (29.2%), pT1 in 57 (51.8%) and pT2 in 21 (19.1%). Regarding nuclear grading, 60 cases (54.5%) were low grade (low malignant potential and low grade) TCC. PCR for general HPV primer was positive in 40 (36.4%) of bladder tumor tissue specimens. Twenty five (22.7%) and 27 (24.5%) of subjects had positive results for HPV 16 and 18 subtypes, respectively. Thirty seven (33.6%) of urine specimens were positive for general HPV primer using PCR and HPV16 and 18 subtypes were positive in 17 (15.5%) and 14 (12.7%) of urine specimens, respectively.

Table 1 shows the association between patient characteristics and grade and stage of bladder tumor. Our data showed no relation between age, sex, cigarette smoking and grade and stage of bladder TCC. Although history of BCG therapy was associated with histological grade of bladder TCC (P = .019). There was not any association between history of BCG therapy and PCR for general HPV primer in bladder tumor tissue (P = .23).

Table 1. The association between patient characteristics and grade and stage of bladder tumor.

Association between stage and grade of bladder TCC and HPV, HPV 16 and 18 infections are shown in **Table 2**.

DISCUSSION

Bladder cancer is one of the most common cancer in the worldwide[13]. Therefore, management of predisposing factors, early detection and appropriate treatment of bladder cancer is crucial for improved patient prognosis and survival[14-17]. Correlation between bladder tumors and HPV infection first reported in 1988 by Kitamura[18]. Afterward, many studies have been designed to evaluate the association between HPV infection and urinary tract neoplasms with controversial conclusions. It has been shown that detection of HPV DNA is largely dependent on a series of technical factors such as tissue fixation, DNA preparation and amplification conditions [19].

In our study PCR for general HPV in bladder tumor tissue was positive in 3 (9.4%), 22 (38.6%) and 15 (71.4%) of Ta, T1 and T2 bladder tumors, respectively (P < .001). PCR for HPV16 in bladder tumor tissue was positive in 2(6.3%), 10 (17.5%) and 13 (61.9%) and PCR for HPV18 in bladder tumor tissue was positive in 1 (3.1%), 14 (24.6%) and 12 (57.1%) of Ta, T1 and T2 bladder tumors, respectively (P < .001, P < .001).

Tenti et al.[7] revealed overall rate of HPV DNA of 32.9%, and the prevalence of HPV 16 and/or HPV 18 infection was significantly higher in low-grade than in high-grade tumors. Lopez-Beltran et al.[20]) studied the samples of a small group of 76 consecutive patients with TCC and determined HPV infection by PCR using DNA primers for HPV types 6, 11, 16, and 18 only. They found a correlation between higher grades of bladder cancer and HPV 16 DNA.

In another study, Furihata et al.[11] studied 90 patients with TCC and determined the presence of HPV DNA types 16, 18, and 33 by in situ hybridization. They showed a significantly worse tumor behavior and survival in patients with tumors positive for HPV DNA and/or p53 protein. In year 2007, Moonen and colageous[9] suggested a positive trend in the correlation between tumor grade/stage and high-risk type HPV infection.

Melchers and coworker demonstrate that HPV can be transported by the urine, probably in exfoliated HPV-infected cells. A similar mechanism may occur during ejaculation, allowing sexual transmission of HPV viruses harbored in the ceils of the male genital tract[21]. Forslund et al.[22] showed that the frequency of HPV DNA-positive urine samples is lower than that of urethra or cervix samples collected in parallel. He also revealed fair to excellent agreement between HPV DNA results for urine and urethra specimens and poor to fair agreement for parallel cervix specimens. In another study, the sensitivity of the urine PCR assay for detecting cervical HPV infection among inner city adolescents was 82%[23].

As we found in our study, there was fair agreement between general HPV primer DNA detection in urine and bladder tumor tissues of patients with TCC. However, the relationship between urine HPV 16 and 18 DNA and bladder tumor tissues of these patients were not as good as general HPV.

Investigations show that HPV may have a prominant role in progression of TCCs to higher stages and/or grades by Inactivation of the tumor suppressor pRB, abnormal p53 protein accumulation, centrosome amplification and lagging chromosomes during mitosis and other unknown mechanisms[24,25].

The most important limitation of this research is the lack of examination of radical cystectomy specimens. Another limitation is the inadequate follow-up of the patient and the evaluation of the tumor's behavior in response to various treatments.

CONCLUSIONS

The prevalence of HPV DNA in patients with bladder TCC is relatively high and associated with higher stage and grade of the disease; so, it can be used as a predictor

of bladder tumor behavior.
Urine sampling for HPV detection is noninvasive and simple approach and is as reliable as tumor tissue sample which could be considered for prognostic and follow up implications.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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