

Association Between Human Papillomavirus and Transitional Cell Carcinoma of the Bladder

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Purpose: Bladder carcinoma is one of the most common malignancies in worldwide. Among several risk factors, Human Papilloma Virus (HPV) have been presumed to play a causative role in the etiology of bladder cell carcinoma. The aim of this study was to evaluate the involvement of HPV infection in biopsy specimens of patients with transitional cell carcinoma at the west of Iran.

Materials and methods: In this study, 97 biopsy specimens including 67 patients with transitional cell carcinoma (TCC) of bladder and 30 cases of control group with the mean age of 63 years were studied using immunohistochemistry to identify HPV.

Results: 22.4% of patients with TCC of bladder and 3.3% of control group were positive for HPV with a meaningful relation ($P=0.019$). The prevalence of HPV was 4.3 fold higher in men than women. Most TCC patients belonged to grades II and III.

Conclusion: Considering the higher incidence of HPV positivity in patients with TCC of bladder compared to control group, it seems to be a meaningful association between HPV infection and TCC of bladder, at least in the west of Iran.

Keywords: biopsy; bladder carcinoma; immunohistochemistry; HPV; transitional cell carcinoma.

INTRODUCTION

Bladder carcinoma as a leading cause of death in worldwide, arises from urothelial epithelium⁽¹⁻³⁾. Transitional cell carcinoma (TCC) is the most common type of bladder carcinoma which represents 90% of bladder malignancies and is commonly associated with cancers of other parts of the urinary tracts such as kidney, ureters and urethra. Bladder cancer is the fourth most common in men, but is less common in women^(4,5). Several risk factors have been considered to be important in development of bladder carcinoma including smoking, certain industrial exposure, arsenic in drinking water, chronic irritation as well as bacterial and viral infection^(6,7). Among them, the causative role of human papilloma virus (HPV) infection has been evaluated in several studies⁽⁸⁻¹²⁾. It has been suggested that HPV is the most important risk factor for development of carcinoma in urogenital system⁽¹³⁻¹⁵⁾. The oncogenic types of HPV, HPV16 and HPV18, have been identified in invasive carcinoma of genital areas. Since the proliferative cycle of HPV is dependent to sequential phases of differentiation of epithelial cells, HPV possesses a particular tendency for epithelial cells with the expression of its oncogenes and pathogenesis^(16,17). In addition, neoplastic alterations in urothelium have several stages in which carcinogenic initiator agent via modification of DNA leads to malignancy. In this line, several studies have suggested the involvement of HPV infection in the development of bladder carcinoma, especially tran-

sitional cell carcinoma⁽¹⁸⁻²¹⁾.

Given that HPV is not able to grow in cellular cultures and also antibody tests are only available in research laboratories, several methods including PCR, in situ hybridization and appropriate immunohistochemical (IHC) staining have been developed to confirm HPV infection in bladder carcinoma tissues. In this study, we aimed to evaluate HPV infection in samples related to patients with TCC using immunohistochemical staining of paraffin embedded blocks to find the interplay between HPV and TCC.

MATERIALS AND METHODS**Clinical samples**

Specimens were retrieved from paraffin embedded bladder carcinoma tissues stored in laboratory of Imam Reza Hospital medical center, including 67 cases with TCC diagnosis and 30 cases from normal biopsies from 2008 to 2011. The slides prepared from paraffin embedded specimens were stained with the conventional hematoxylin and eosin method. All specimens were reviewed and original diagnoses were confirmed.

Immunohistochemistry

Immunohistochemical evaluation was performed on 5 μ m formalin fixed paraffin embedded tissue sections. In brief, an anti-human HPV mouse monoclonal antibody (clone K1h8, DAKO) was used as the primary antibody at a dilution of 1/30000. Then, secondary antibody con-

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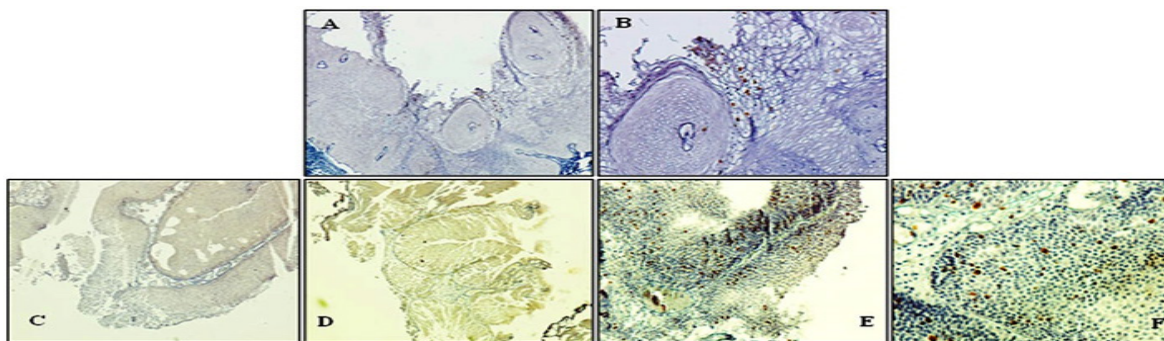


Figure 1. Immunohistochemical staining for human papillomavirus (HPV) in bladder cell carcinoma. Candyloma section (Positive-HPV) as control with magnifications of 20X and 40X (A; B), HPV negative in TCC with magnifications of 20X and 40X (C; D) and HPV positive in TCC with magnifications of 20X and 40X (E; F) indicated by nuclear and cytoplasmic staining of tumor tissues.

jugated with horseradish peroxidase and diaminobenziden chromogen were added to identify the presence of HPV in samples. Candyloma specimen was used as a positive control. Nuclear staining of tumoral cells in TCC specimens and urothelial cells in normal specimens were considered as positive samples.

Statistical analysis

Statistical analysis was performed by SPSS statistical software package version 16, using *T*-test and chi-square tests.

RESULTS

In this study, immunohistochemistry was performed on paraffin embedded bladder TCC tissues to distinguish the involvement of HPV infection in development of carcinogenicity of bladder (Figure 1). From the population of 67 TCC specimens and 30 normal biopsy specimens, 15 and 1 cases, respectively, were positive for HPV infection. There was a meaningful relation between TCC carcinogenicity and HPV infection ($P = .019$) (Figure 2).

Among TCC patients, 86.6% (58 cases) were male and 13.4% (9 cases) were female. In control group, 56.7% (17 cases) were male and 43.3% (13 cases) were female. As shown in Figure 3, there was not meaningful relation between gender and HPV infection ($P = .933$). The histological grading of tumor specimens indicated

that 1 case with grade 1, 24 cases with grade 2, 32 cases with grade 3 and 10 cases with grade 4 were found in 67 TCC specimens according to WHO/ISOP classification (Figure 4A). Among them, immunohistochemical analysis showed that 5 cases of grade 2, 4 cases of grade 3 and 6 cases of grade 4 were positive for HPV nuclear staining. According to Figure 4B, there was no meaningful relation between the grade of TCC and HPV infection ($P = .718$).

DISCUSSION

The involvement of human papillomavirus in bladder carcinoma has been reported by several studies^(18,22). In this line, the high prevalence of HPV infection in patients with TCC indicated the possible association of HPV and carcinogenesis of transitional cell carcinoma of bladder⁽²³⁻²⁶⁾. In this study, we aimed to investigate the meaningful relationship between HPV and patients with transitional cell carcinoma. We studied the expression of HPV in 67 patients with TCC and 30 normal samples as control using immunohistochemistry. 15 cases (22.4%) of TCC patients' group and 1 case (3.3%) of control group were found to be positive for HPV infection.

The first report about the association of HPV and bladder carcinoma was reported by Kitamura based on southern blot analysis in 1988⁽²⁷⁾. After that, several

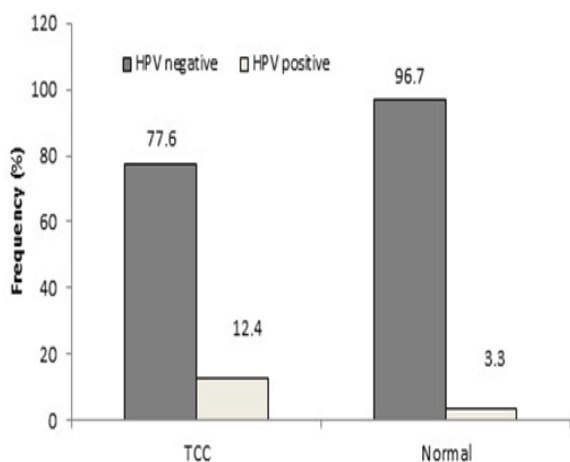


Figure 2. Infection pattern of HPV in bladder biopsy specimens among patients and control groups.

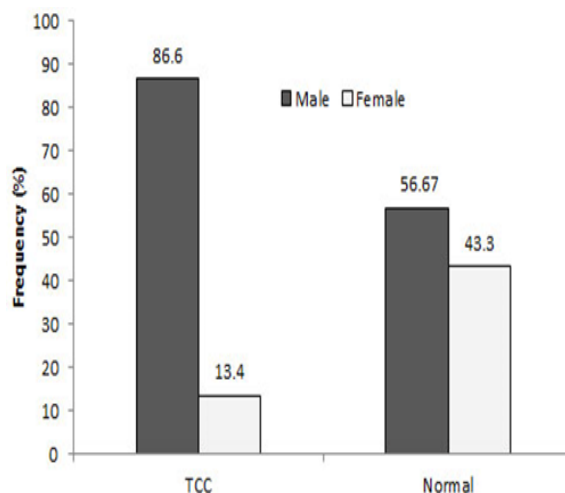


Figure 3. The incidence of sex in bladder biopsy specimens among patients and control groups.

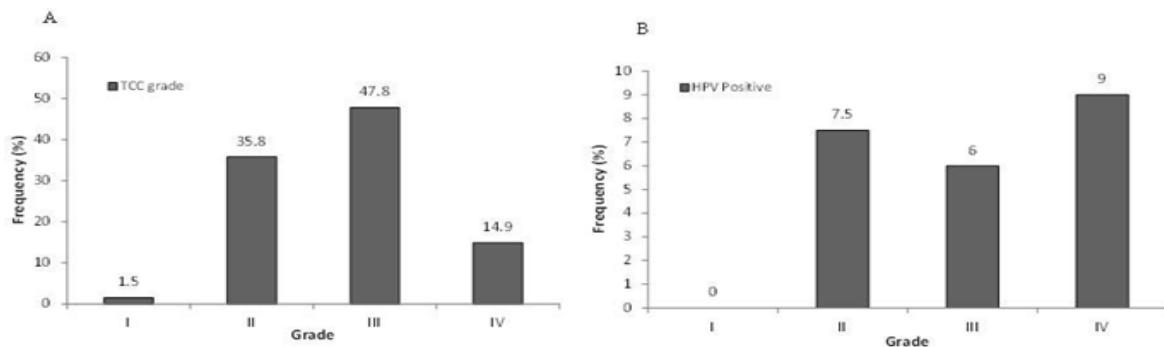


Figure 4. Histological grading of urinary bladder carcinoma in bladder biopsy specimens among patients (A). Relation between HPV positivity and histological grading of TCC patients (B).

contradictory studies have been reported about the involvement of HPV in carcinoma of bladder. Lopez-Beltran et al. assessed the role of HPV in TCC using IHC and ISH methods. They identified 25 cases with HPV infection using IHC and 12 cases with positive HPV-DNA positive using PCR in 76 patients with TCC⁽²⁸⁾. In another study, Youshya and co-workers studied the involvement of HPV in 76 patients with TCC using PCR and IHC methods. Interestingly, different results were acquired from two methods. 47 cases were positive for HPV according to IHC while none were positive for HPV-DNA by PCR. Therefore, they suggested that there were no possible association between HPV infection and carcinogenesis of TCC⁽⁹⁾. Shigehara et al. evaluated the etiologic role of HPV infection in bladder carcinoma. They studied the involvement of HPV in 244 cases of patients with bladder cancer during 1997 to 2007. They analyzed the expression of HPV-DNA in frozen specimens of TUR bladder tissue with PCR and HPV-L1 capsid protein of HPV using IHC. Therefore, it seems that high risk types of HPV leads to carcinogenesis of low grade bladder cancer in juvenile patients⁽¹⁹⁾. Another controversial study about the involvement of HPV in urothelial cancer was performed by Yavuzer et al. They studied 70 cases of urothelial carcinoma to find HPV using PCR with respect to 18 cases of patients with cervical carcinoma as control. They found that there was no association between HPV infection and bladder urothelial carcinoma⁽²⁹⁾. In another study by Barghi et al., 58 cases of TCC specimens and 20 normal cases as control was evaluated using PCR to identify HPV-DNA. They found that the possibility of HPV involvement in TCC is related to geographical regions⁽¹⁵⁾. Selma et al indicated the interplay of HPV and TCC in Tunisia. They examined 119 patients with TCC, 5 cases with squamous cell carcinoma and one case with adenocarcinoma by PCR based method to distinguish different subtypes of HPV. Their results showed that anogenital HPV infection seems not to play a significant role in pathogenesis of bladder carcinoma⁽³⁰⁾. Eslami et al. considered 147 cases of TCC patients and 39 cases of normal specimens as control to study the relation between HPV infection and TCC. They used PCR to identify HPV-DNA in patients. They found that there is no meaningful association between HPV infection and grade of bladder tumor; while it seems that genital HPV infection especially subtype 18 augmented the risk of bladder carcinoma in Iranian population⁽³¹⁾.

CONCLUSIONS

Taken together, our present study indicated that there was a meaningful association between HPV infection and transitional cell carcinoma. However, we could not find any meaningful relation between grade of TCC, age and sex of patients and the presence of HPV.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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