Original Article

Association between the Evidence of Human Papilloma Virus Infection in Bladder Transitional Cell Carcinoma in Men and Cervical Dysplasia in their Spouses

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Abstract

Background: Cervical cancer is one of the most common tumors in women. One of its risk factors is direct contact with viruses, in particular human papillomavirus (HPV). Recent studies have shown a prevalence of 2%-35% for HPV in cases with bladder cancer. In addition, some malignancies of the urogenital organs in males may promote the probability of cervical cancer in their spouses. In this study, the relationship between cervical dysplasia in women and evidence of HPV infection in tissue specimens obtained from their spouses' bladders has been investigated.

Methods: This cross-sectional study was conducted on 82 male patients with bladder tumors and their spouses between February 2004 and February 2007 in Tehran. We gathered data related to the histopathology of the transitional cell carcinoma (TCC) in men and Pap smear tests of their spouses. Tissue specimens of patients with bladder TCC were analyzed for HPV infection using polymerase chain reaction (PCR). HPV-positive specimens were tested for subtypes 16 and 18.

Results: In 24 (29.3%) men, bladder tumor samples were positive for HPV infection. Of these, we found HPV-18 infection in 9 (37.5%), while 3 (12.5%) were infected with HPV-16. In the spouses of those men with HPV-infected bladder tumors, 4 (4.9%) cases had cellular dysplasia noted on their Pap smear tests. We found no dysplasia in those women whose husbands had bladder TCC, but no HPV infection ($P = 0.006$).

Conclusion: It is possible to decrease the incidence of bladder TCC in men and cervical cancer in women through public education regarding the methods of transmission and avoidance of risky sexual behaviors.

Keywords: Bladder tumor, cervical dysplasia, human papillomavirus

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Introduction

Bladder cancer is estimated for around two-thirds of all urinary tract cancers. It is the ninth most common cancer in the world, and the fourth most common cancer among Iranian males.[1] Recent studies have shown a prevalence of 2% – 35% for human papillomavirus (HPV) infection in cases with bladder cancer.[2] By using polymerase chain reaction (PCR), a study from Iran has demonstrated the presence of HPV in 35.6% of bladder transitional cell carcinoma (TCC) tissue specimens, which was seven times higher than the control group.[3]

Cervical cancer is one of the most common cancers in women. Viral agents are the main risk factors for the development of cervical cancer. Owing to the cervical cytological screening program (Pap smear), cervical dysplasia or cancer is diagnosed earlier and overall survival has improved. The sensitivity of the Pap smear test for the diagnosis of cervical dysplasia or cancer has been estimated to be 80%, and the prevalence of HPV infection in cervical interepithelial neoplasia appears to be around 90%.[4]

A strong relationship between cervical dysplasia found on the Pap smear test and the evidence of HPV infection,[5] as well as an association between bladder TCC and HPV infection[4] have been demonstrated. According to research, some malignancies of the urogenital organs in males may promote the probability of cervical cancer in their spouses. Rosemborg et al. have reported an observed risk of cervical neoplasia in the spouses of males diagnosed with bowenoid papulosis penis which is associated with HPV-16 infection.[6] We have studied the incidence of cervical dysplasia in spouses of males with bladder TCC considering the evidence for HPV infection in bladder tissue specimens.

Our cross-sectional study was carried out on 82 males with bladder TCC who were admitted to three general hospitals (Shohada, Labbafinejad, and Modarres) in Tehran, Iran between February, 2004 and February, 2007.

Materials and Methods

This study was performed on males with bladder TCC. Exclusion criteria were as follows: presence of any other types of benign or

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Table 1. Frequency of human papillomavirus infection (HPV) in tumors from 82 men with transitional cell carcinoma (TCC) of the bladder.

	Number (%)	Mean age (SD)	*P*-value
HPV negative TCC cases	58 (70.7%)	66.8 ± 7.7	(-)
HPV positive TCC cases	24 (29.3%)	64.6 ± 7.6	0.25
HPV-16 positive TCC cases	3 (3.6%)	5.5±66.6	0.41
HPV-18 positive TCC cases	9 (10.9%)	65.7 ± 6.0	0.40

Table 2. Characteristics of women with and without dysplasia in their Pap smears.

	Dysplasia	No dysplasia	*P*-value
Average age (years)	60.5 ± 8.8	59 ± 5.2	0.73
Average period of intercourse (years)	38 ± 1.8	39.7 ± 9	0.2

Table 3. Prevalence of cervical dysplasia in spouses of bladder of transitional cell carcinoma (TCC) cases.

	Without cervical dysplasia (n=78)	Cervical dysplasia (n= 4)	*P*-value
Men with TCC not infected by HPV (n=58)	58 (100%*)	0 (0%*)	0.006
Men with TCC infected by HPV (n=24)	20 (83.3%*)	4 (16.7%*)	
* Row percentages			

malignant bladder tumors, ambiguous pathological results, insufficient tumor tissue for DNA extraction after the removal of paraffin, and patients whose spouses were either unavailable or unwilling to undergo Pap smears.

Pathological files and slides of tumor tissue following transurethral resection of the tumor (TURT) were studied to detect the presence of the bladder tumor. Slides of positive samples were studied by a single pathologist to ensure the availability of sufficient tumor tissues. After removal of the paraffin, tumor tissues were placed in specially sealed tubes under appropriate conditions to prevent contamination. Later, all specimens were sent for PCR analyses.

Pap smear

Pap smear tests were carried out on the spouses of our subjects according to WHO standards, using approved devices. All Pap smears were analyzed and reported by one pathologist.

Molecular analysis

Existing tissue from the tumor areas marked on the paraffin block were precisely separated by a surgical blade (minimum: 3 × 3 mm). Excised tissues were placed in a grill for five hours in order to dissolve any remaining paraffin. Next, the separated tissue was placed in lysing buffer to be digested for four hours at 37°C. After tissues were in boiling water for 20 minutes, both the tissue and viral DNA were extracted by phenol and chloroform. The DNA was extracted by centrifuge (Hettich Company). Extracted DNA was concentrated by absolute ethylic alcohol to render it ready for PCR.

PCR reaction and DNA transcription

In this reaction, 0.1µg DNA, 20 picomoles of each of the primers (general and specific for HPV 16 and 18), 2 mmoles of each of the four deoxynucleotide triphosphates, 15 mmoles of $MgCl_2$, and 2 units of DNA polymerase enzyme were placed in specialized PCR tubes. The reaction was carried out in a thermal shaker (Personal model, Eppendorf, Germany) according to the following schedule: denaturation of two DNA strands at 94°C for 30 seconds.; annealing 60°C for 30 seconds; and extension at 72°C for 30 seconds (this stage was repeated 30 times). After completion of the reaction, the product was placed on a 2% agarose gel for electrophoresis (Akhtarian Company) after which it was stained by Yttrium Bromide and examined by a UV transilluminator (UVI Tech, England). If the sample was positive for HPV virus, the DNA bands were visible.

Statistical analysis

Data were entered into SPSS version15 software and analyzed according to the goals of the study. Associations were analyzed by the chi square test. *P*-values < 0.05 were considered statistically significant.

Results

Average age for men was 66.2 ± 7.6 years and their spouses, it was 60.5±8.9 years. According to the t-test, there was no significant difference between the ages of HPV-infected and non-HPV-infected men ($P = 0.25$). There were no significant differences between both groups of men in terms of age ($P = 0.73$) and period of intercourse ($P = 0.2$; Tables 1 and 2).

HPV-infected bladder tumor samples were detected in 24 (29.3%) men. Results from spouses of the men with bladder tumors showed that 4 (4.9%) spouses had evidence of cellular dysplasia in their Pap smears; their husbands were HPV-positive. No dysplasia was noted in women whose husbands had TCC, but were HPV-negative ($P = 0.006$; Table 3). There were 9 (37.5%) HPV-infected bladder tumors reported as HPV-18 and 3 (12.5%) that were HPV-16 (Table 1).The rate of dysplasia in the Pap smear results of women whose husbands were infected with HPV-16 and HPV-18 were both 33.3%.

Discussion

TCC is responsible for 90% of bladder malignancies. Recently, different findings have been reported on the relationship between HPV infection and TCC.[7–11] However, there is a strong association between HPV infection and invasive cervical cancer.[4] Male partners may markedly participate in the development of cervi-

cal cancer in their female partners by being carriers of oncogenic HPVs. There is a higher prevalence of HPV infection that has been reported in bladder cancer when compared to controls.[3]

The sexually transmitted nature of cervical squamous neoplasia has been strongly suggested by epidemiological studies. This has led to the theory that a male at high risk can place his female partner at an increased risk for cervical neoplasia.[12]

In our recent study, the prevalence of HPV-16 among males with bladder TCC was 12.5%, the prevalence of HPV-18 was 37.5%, and the total prevalence was 29.3%. In a comparison of these two carcinogenic types, HPV-18 was far more prevalent. These findings were similar to those by Barghi et al. who researched the relationship between bladder TCC and HPV. The results of their study showed a higher percentage of bladder tumors were seen in patients infected with HPV (35.6%), of which HPV-18 was the most common subtype (66.7%).[3]

The prevalence of dysplasia in Pap smear tests performed for cervical cancer screening in the US has been reported as 3% – 6%,[13] while this rate in the Iranian cities of Yazd was 0.13%[14] and in Isfahan[15] it was 0.65%. In a study of cytologic examinations from Southern Iran, the prevalence rate of invasive cervical cancer among 35222 women screened for the first time was 2.3 per 1000.[16] In our study the prevalence of cervical dysplasia in the spouses of the men with bladder tumors was 4.9% which was elevated compared to the rates in Iran . This could be a sign of exposure to HPV infection in some of the women in this group (spouses of men with bladder tumors).

One study was performed to find the frequency of squamous cell carcinoma of the cervix between wives of 889 men diagnosed with epidermoid carcinoma of the penis. Among wives of men with penile carcinoma, there were eight cases of cervical carcinoma in contrast with none among the control wives.[17]

In a previous study that investigated the role of men in cervical cancer, populations at high (Colombia) and low (Spain) risk for cervical cancer were interviewed. The researchers concluded that there was a higher prevalence of penile HPV DNA in husbands of women with cervical dysplasia than in husbands of the control group.[18]

In the current study, we have shown the presence of cervical dysplasia in 16.7% of women whose husbands suffered from bladder TCC and were infected with HPV. By taking this elevated rate into consideration, we recommend that all patients with bladder TCC should be tested for HPV infection and their spouses monitored for cervical dysplasia. If the tumor sample is HPV-positive, the spouse should be periodically tested for the presence of cervical dysplasia. Vaccination against HPV should be considered in these individuals.

Until now, there has been no reported study about the prevalence of cervical dysplasia among the spouses of men with bladder TCC. Our study showed that cervical dysplasia among the spouses of men with bladder tumors who were HPV-positive was significantly higher compared with those whose tumor tissues were HPV-negative.

Our findings probably indicate that transmission of HPV from the urinary tract of males with bladder tumors to their spouses may be a predisposing factor for the induction of cervical dysplasia in their spouses. Therefore, avoidance of risky sexual behavior and the use of barrier methods are important ways to reduce the risk of cancer in both sexes.

These findings warrant more frequent, careful examinations for wives of men with bladder tumors in order to monitor for cervical dysplasia. Additionally, vaccination for HPV in these women should be seriously considered.

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