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Original Article

Cytokeratin 20 in Transitional Cell Carcinoma of Bladder and Its Relation with Prognostic Factors

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ABSTRACT

Background and Aims: **Immunohistochemical tests are one of the most important tests, which are under study to determine the prognosis of the cancers such as transitional cell carcinoma. By the time, flexible cystoscopy and urine cytology are the routine tests for following up the patients with transitional cell carcinoma, which are both operator dependent. On the other hand, cystoscopy is an invasive method, and urine cytology is a method with low sensitivity. The aim of this study was to determine CK20 in patients with transitional cell carcinoma of bladder and its relation with prognostic factors, which are the stage and the grade of the tumor.**

Materials and Methods: **Our study was done in Mostafa Khomeini Hospital from 2007 to 2010 on the 2001 to 2009 stored information files, included 53 patients diagnosed as transitional cell carcinoma of bladder (TCC) of different stages and grades that were underwent total cystectomy. Immunohistochemical staining was performed on tissue sections with specific CK20 antibody. Then the samples were studied by light microscope so positive and negative cases were identified.**

Results: **According to statistical analysis there were significant reverse relationship between CK20 and stage, and significant reverse relationship between CK20 and grade (*P*= 0.000).**

Conclusion: **Immunohistochemical study of patients with transitional cell carcinoma of bladder in order to identify CK20 can be a useful method to determine the prognosis of these patients.**

Keywords: **Cytokeratin 20, Bladder Cancer, Prognosis**

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Introduction

Cancers are the second cause of morality in The United States after cardiovascular events (1). Cancers may occur in any site of human's body such as genitourinary tract. The walls of genitourinary tract are composed of transitional cell epithelium under which lies loose connective and elastic tissue. Ninety percent of urogenital malignancies occur in bladder, 8% in calices, and 2% in ureters and urethra (2). Therefore, the bladder carcinoma is the most common malignancy in genitourinary tract. Ninety eight percent of them are epithelial cancers which most of them are transitional types (3).

Bladder cancer is the fourth common cancer in men and 10th in women. The incidence of bladder cancer in men is three times more than women are. The average age of diagnosis is 65. Besides, approximately 85% of them are localized to the bladder and 15% have spread to distant sites (3).

Bladder cancer is the fifth most common cancer in Iran. The incidence is 1.6 in every 100,000 person and in men is 4 times more than women are (4).

The bladder transitional cell carcinoma (TCC) has more mortality than TCC in the other sites of the genitourinary tract because it has less signs and symptoms due to its anatomic position in comparison with upper tumors, which may cause obstruction, or other symptoms. So bladder tumors are usually diagnosed in higher stages and grades (5).

Immunohistochemical assessment of cytokeratins is one of the newest tests, which is proposed to determine the prognosis of patients diagnosed as bladder cancer. Cystoscopy and urine cytology, as common methods to follow up these patients, are both highly dependent on the skill of the operator , in addition cystoscopy is an invasive method and cytology has low sensitivity and is not reproducible (1).

Cytokeratins are the major structural proteins in the cytoplasm of epithelial cells and their derivatives. Keratin filaments are built from lateral and longitudinal interactions involving type I-II heterodimers (6). Each type of epithelial cells synthesizes at least one type I and one type II keratin, which co polymerize into filaments (7). The organization of keratin filaments and their association with plasma membranes suggest that their principal function is structural to reinforce cells and to organize cells into tissues. Keratin filaments are characterized by tissue-specific expression patterns from early embryogenesis onwards, suggesting that these proteins are important in defining tissue structure and potential function (8). Keratins also influence the availability of regulatory molecules such as apoptosis-including factors, heat shock proteins or signaling molecules and so, affect the sensitivity of cells to proliferative and apoptotic stimuli and play a role in cellular stress responses and drug resistance (9).

Keratin 20 is the most recently identified type I keratin protein of 46 KD, which shows a limited pattern of expression in normal tissues (10). The expression of CK20 in urothelium was restricted to superficial umbrella cells even in the presence of severe inflammation. Only malignancy induced alteration in CK20 expression pattern. The pattern of CK20 immunohistochemical staining is a useful adjunct morphology in the diagnosis of urothelial dysplasia, since only malignant cells will show CK20 immunostaining (11) .

The aim of is study was to determine CK20 in patients with transitional cell carcinoma of bladder and its relation with prognostic factors which are the stage and the grade of the tumor.

Material and Methods

This cross-sectional study included 53 patients diagnosed as bladder TCC of different stages and

grades that were underwent total cystectomy.

The cases were selected from the Mostafa Khomeini Hospital surgical pathology files from 2001 to 2009.

Tumor samples were formalin-fixed for 24-48 hours and embedded in paraffin wax. Three µm sections were cut from paraffin blocks and immersed in water and alcohol for 5 minutes, then heated in 60^0C for 30 minutes. After that dehydration and deperoxidation were done using xylol, alcohol 100%, alcohol 96%, and then alcohol 75% and at the end water each one for 5-10 minutes. In the next step, we washed the samples with phosphate buffered saline (PBS) 10% three times and used H2O2 and methanol to redeperoxidation for 10 minutes, and then we washed them with PBS 10% for the second time. Then we heated them in 120^0C and rewashed them in PBS 10%.

At the end, immunohistochemical staining was performed on tissue sections with histostain and specific CK20 antibody. Then the samples were studied by light microscope.

The stage and the grade of each case were assessed by an expert pathologist and then CK20-positive and CK20-negative cases were identified. Immunohistochemical staining was performed used of specific CK20 antibody (Novacastra , England) .

Statistical analyses: We used SPSS and Chai – square statistical method to analyze the data.

Results

This study was performed on 53 patients. Forty-five (84.9%) patients were men and eight (15.1%) of them were women. CK20 immunohistochemichal study was positive in 30 patients and negative in 23.

Twenty-two cases (41.5%) were diagnosed as stage III that was the most common stage. Other patients fell into each stage I, IIa and IIb (9 in stage I, 8 in IIa and 14 in IIb) .

In the CK20 positive group (30 cases) , 9 (30%) were diagnosed as stage I which was the most common stage and 5 cases (16.1%) fell into stage III which was the least common stage. In the CK20 negative group (23 cases), 17 cases (73.9%) were diagnosed as stage III (most common) and none of them were in stage I and IIa. Table 1 shows an overview of frequency of cases in different stages in two groups separated by CK20 status. According to the table, all the patients diagnosed as stage I or IIa were CK20 positive. In stage IIb, 8 cases (57.14%), and in stage III, only eight cases (21.73%) were positive.

According to these results and statistical analyses, there was a significant reverse relationship between stage and CK20. It means the less detection of CK20 in higher stages (*P*=0.00).

Table1- Frequency of different stages of TCC in the patients who had underwent hysterectomy

Stage / Cytokeratin20	Stage I	IIa	IIb	III	Total
Positive	9	8	8	5	30
Negative	0	0	6	17	23
Total	9	8	14	22	53

We also studied the relation between grading and CK20. Twenty-five cases (47.2%) were diagnosed as grade 3, which was the most common grade, 7.5% as grade 1, 30.2% as grade 2 and 15.1% as grade 4.

In cytokeratin positive group (30 cases), 14(87.5%) were identified as grade 1 which was the most common grade and none of the cases were identified as grade 4. In the cytokeratin negative group (23 cases) , none of them were grade 1 and 8 cases(34.7%) were identified as grade 4 which was the most common grade. Table 2 presents and overview of frequency of cases in different grades in two groups separated by cytokeratin status.

According to Table 2, all the patients identified as grade 1 were CK20 positive. In the grade 2 group, 87.5% of them and in the grade 3 group, 48% of them were CK20 positive and in grade 4 group, all the patients were CK20 negative.

According to these results and statistical analyses, there was a significant reverse relationship between grade and CK20. It means the less detection of CK20 in higher grades (P=0.00).

Table 2- Frequency of different grades of TCC in the patients who had underwent hysterectomy

Cytokeratin20 \ Grade	1	2	3	4	Total
Positive	4	14	12	0	30
Negative	0	2	13	8	23
Total	4	16	25	8	53

Discussion

Data from the current study agrees with less detection of CK20 in higher grades and stages. So according to these results and comparing them with other studies, we found that immunohistochemical detection of cytokeratin has the potential to determine the prognosis of patients in different stages and grades of TCC.

In the study by Morsi MI *et al*. aimed to evaluate CK20 immunostaning in 100 bladder cancer patients in comparison to cytology and PCR, resulted no relation between CK20, which is different to our study. The different types of study, different number of patients in 2 studies and of course different methods of detecting CK20 may justifies the different results of these two studies (12).

Of course, we think that immunestaining study is more reliable than cytology according to the function of these proteins, which are important in defining tissue structure. Therefore, cells in urine sample do not seem to have enough reliability for cytokeratin studying (12).

In the study by Parker DC *et al*. aimed to evaluate a panel of tumor markers containing CK20, proposed using other tumor markers specially thrombomodulin and high molecular weight cytokeratins along with CK20 in order to increase sensitivity and specificity and decrease false positives and negatives. Also this study emphasizes on using these tumor markers for screening and diagnosing more than determining patients' prognosis (13).

In the study by Elsalahi in Egypt, which aimed to detect the expression of CK19 and 20 in tissue specimens of bladder cancer patients, the results were correlated with clinic-pathologic parameters, bilharziasis and the occurrence of relapse of the carcinoma among Egyptian bladder cancer patients. It showed significantly correlation to advanced stage and grade and strongly associated with malignant phenotype of Egyptian bladder tissues, so they may be used as

additional markers for assessment bladder cancer patients (14).

There is an important detail about overlapping of SCC and TCC in tumor markers' expression, so more studies is needed to differentiate these two cancers and determine expression of tumor markers in order to differentiate them with tumor marker panels especially in endemic areas for bilharziasis, because it accounts for both these two cancers as a risk factor (14).

Other several groups also have done some studies to detect CK20 with or without other tumor markers in order to diagnose, screen, or determine patients' prognosis. CK20 is an important biomarker, which can be used to identify TCC especially in cases whose malignancy is not confirmed by morphology alone (15). CK20 immunocytology is more sensitive than standard cytology in the detection of TCC especially in T1, grade 2 and grade 3 tumors. It proposed immunoassay might progressively replace conventional cytologic screening in the diagnosis of bladder cancer. However, unfortunately in this study was not directly mentioned to prognosis, which was the main goal in our study (16). Although these two studies have different methods in comparison to our study, their results do not have any conflicts with ours. The immunohistochemical study of CK20 on urine smears in 169 cases of TCC by Li Hx *et al* showed that this biomarker can be useful in early diagnosis of TCC of bladder (17).

A case-control immunohistochemical study was done on 42 patients diagnosed as TCC and 17 controls on urine samples and showed that immunohistochemical study and urine cytology together can lead to more sensitive and accurate diagnose of TCC and use of other biomarkers such as NMP22 can improve the specificity and sensitivity of immunohistochemical study of CK20(18).

Conclusion

According to the results of our study, the low sensitivity of urine cytology and invasiveness of cystoscopy, immunohistochemical study of patients with transitional cell carcinoma of bladder in order to identifying CK20 can be a useful method to determine the prognosis of these patients.

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